



**INHERITANCE AND CHARACTERIZATION OF COOKING TIME,
SEED IRON AND ZINC CONTENT IN SELECTED AFRICAN COMMON
BEAN GERMPLASM**

BY

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DECLARATION

I, **Ms. Irene Mukai Mughi**, declare that this work has not been submitted for any degree other than that of Master of Science in Plant Breeding and Seed Systems of Makerere University. I also declare that this work is the result of my own investigations except where otherwise stated.

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DEDICATION

To my loving parents, Mr and Mrs Gregory Mbiti, my loving husband George, and our beloved children Remmy and Emerald

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TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDICES	x
ABSTRACT	xi
CHAPTER 1: INTRODUCTION	1
1.1 Background	1
1.1.1 Global production and importance of common bean	1
1.1.2 Production constraints of common bean in Uganda	3
1.1.3 Consumer preferred traits in common bean	3
1.2 Problem statement	4
1.3 Justification	5
1.4 Objectives	6
1.5 Hypotheses	6
CHAPTER 2: LITERATURE REVIEW	7
2.1 Origin, classification and occurrence Common bean	7
2.2 Morphological diversity in common bean	8
2.3 Traits of importance in common bean	9
2.3.1 Cooking time	9
2.3.2 Factors influencing cooking time	11

2.3.3	Genetics of cooking time.....	11
2.4	Importance of Iron and Zinc content in common bean.....	12
2.4.1	Potential for improvement for Iron and Zinc content in common bean.....	13
2.4.2	Genetics of Iron and Zinc content.....	13
2.5	Profile of common bean genotypes in Uganda.....	14
CHAPTER 3: IDENTIFICATION OF COMMON BEAN GENOTYPES WITH SHORT COOKING TIME, HIGH IRON AND ZINC CONTENT		16
3.1	Background.....	16
3.2	Materials and methods	17
3.2.1	Study site.....	17
3.2.2	Genotypes used in the study.....	17
3.2.3	Experimental design and management.....	19
3.3	Data collection	19
3.3.1	Soil sampling and analysis.....	20
3.3.2	Agronomic traits.....	20
3.3.3	Yield data	21
3.3.4	Disease data.....	21
3.3.5	Cooking time estimation	24
3.3.6	Seed Iron and Zinc analysis	25
3.4	Data analysis	25
3.5	Results.....	26
3.5.1	Soil status of experimental site.....	26
3.5.2	Variability in cooking time of the genotypes evaluated.....	26
3.5.3	Variability in water absorption of 152 bean genotypes.....	29

3.5.4	Variability in Iron and Zinc content in the genotypes.....	29
3.5.5	Variability of the genotypes in agronomic, yield and disease data.....	33
3.5.6	Correlation of cooking time, %hydration, Fe and Zn content, diseases and yield.....	34
3.6	Discussion and conclusion.....	35
CHAPTER 4: MODE OF INHERITANCE FOR COOKING TIME, SEED IRON AND ZINC CONTENT IN SELECTED COMMON BEAN GENOTYPES		38
4.1	Introduction.....	38
4.2	Materials and methods	39
4.2.1	Study site and germplasm	39
4.2.2	Population development.....	39
4.3	Data collection and analysis.....	41
4.3.1	Estimations of heritability	41
4.4	Results.....	42
4.4.1	Performance of progeny and parents for cooking time, Fe and Zn content	42
4.4.2	Estimates of general combining ability for cooking time, Fe and Zn seed content ...	43
4.4.3	Estimates of specific combining ability effects for the crosses	44
4.4.4	Heritability estimates	45
4.5	Discussion and conclusion.....	45
CHAPTER 5: GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS		49
5.1	General discussions.....	49
5.2	Conclusions.....	50
5.3	Recommendations.....	51
REFERENCES		52
APPENDIX.....		59

LIST OF TABLES

Table 1 : Common bean germplasm used in the study and their characteristics	18
Table 2: Soil Status of trial site for evaluation of common bean genotypes at Kawanda	26
Table 3: Mean square values for cooking time of bean genotypes evaluated at Kawanda	26
Table 4: Seasonal performance of genotypes for cooking time.....	28
Table 5: Mean squares for water absorption (% hydration) among the bean genotypes evaluated over two seasons in Kawanda.....	29
Table 6: Mean squares for Iron and Zinc content in bean genotypes evaluated over two seasons at Kawanda.....	30
Table 7: Performance of evaluated bean genotypes for seed Iron and Zinc concentration with comparison to the iron check	31
Table 8 : Seed Iron and Zinc concentration in selected bean genotypes per season	32
Table 9: Mean squares for agronomic, diseases and yield data for the 152 evaluated bean genotypes	33
Table 10: Correlation of cooking time, Iron and Zinc, diseases and yield	34
Table 11: Characteristics of ben parents used in inheritance study for cooking time, seed iron and zinc content.	40
Table 12: The mating scheme used for an inheritance study of cooking time, Iron and Zinc seed content at CIAT Uganda.	41
Table 13: Formulae used for estimating heritability and Baker's ratio	42
Table 14: Mean squares values for combining ability for cooking time, seed iron and zinc content in beans	42
Table 15: Mean square values for GCA effects and parental means for cooking time, seed iron and zinc content in beans	43
Table 16: Mean square values for SCA effects	44
Table 17: Bakers ratio, Narrow sense heritability and broad sense heritability estimates	45

LIST OF FIGURES

Figure 1: Range of cooking time for 152 bean genotypes evaluated over the two seasons at Kawanda	27
Figure 2: Average Iron (A) and Zinc (B) content in the evaluated common bean genotypes over two seasons	31
Figure 3: General combining ability estimates for the evaluated parents for cooking time, seed iron and zinc content	43

LIST OF APPENDICES

Appendix A: Summary table of means agronomic, cooking time, iron and Zn content, %hydration, yield and disease data for the 152 genotypes evaluated for two seasons at Kawanda	59
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ABSTRACT

Long cooking time for beans continues to be a major hindrance to the widespread consumption of beans. Prolonged cooking time leads to structural changes at the grain cellular level, resulting in a loss of nutrients such as Iron (Fe) and Zinc (Zn) which are important nutrients in addressing micronutrient malnutrition (“hidden hunger”). The aim of this study was to evaluate the diversity for cooking time, iron and zinc content in a total of 152 genotypes from around eastern Africa, including Kenya, Uganda, Tanzania, Ethiopia, and Rwanda, and to determine the mode of inheritance for cooking time, Fe and Zn content in common bean genotypes.

A total of 152 common bean genotypes released by the Pan-Africa Bean Research Alliance (PABRA) across Eastern Africa were planted in the field at International Centre for Tropical Agriculture (CIAT) farm at Kawanda, 13km from Kampala city, during two rainy seasons of 2015 B (April – July) and 2015D (September – December). Data collected included soil nutrient composition for the site used in each season, agronomic data and disease data.

Six parental genotypes were crossed in a screen house at CIAT-Kawanda, using a 6 x 6 half diallel mating design. The F1's were advanced to F2 generation which was subjected to cooking time, Fe and Zn content tests. Cooking time test was carried out at CIAT-Kawanda on plot basis using the standardized Matson cooker method. Fe and Zn analysis was carried out at Rwanda Agricultural Board (RAB) research station in Rubona using the X-Ray Fluorescent (XRF) platform.

Across the two seasons, among the 152 genotypes studied, 5 had a cooking time of <45 minutes, 55 genotypes cooked for 46-60 minutes and 92 genotypes cooked for >61 minutes. In response to Fe and Zn seed content, 8.7% were high in Fe (>70 mg/kg) whereas 69.1% were high Zn (>30 mg/kg). A total of 15 genotypes (Amahunja, Awash melka, Bihogo, CAB 2, ECAPAN021, G858, Icaquimbaya, KK20, NABE12C, NABE4, NABE6, ROBA-1, RWR1873, RWV3006) were consistent in short cooking time for the two seasons and had a Fe content above the low Fe check (CAL96 – 55mg/kg). Analysis of variance showed a highly significant variation among genotypes, general combining ability (GCA) and specific combining ability (SCA) components in the F2 for cooking time, Fe and Zn content, indicating that additive variance predominated with non-additive gene effects contributing a considerable amount of variations.

Awash melka had the desired GCA combination for cooking time, Fe and Zn content. Significant SCA effect for cooking time and high Fe content were observed in NgwakuNgwaku x KATX56 identifying it as a good cross for combining short cooking with high Fe and Zn content.

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Global production and importance of common bean

Common bean (*Phaseolus vulgaris* L) is an important legume food crop for over 700 million people worldwide (Broughton et al., 2003). In 2014, the leading producers of the crop were India (3,303Mt), Brazil (2,990 Mt), Myanmar (2,040 Mt), China (1,539 Mt) and United States of America (1,197 Mt). In Africa, major producers include Central Africa (DR Congo, Rwanda and Burundi) and Eastern Africa (Kenya, Uganda, and Tanzania). Eastern Africa has the highest bean production in sub-Saharan Africa at 1,297,000 tonnes per annum. Production volumes in Uganda, Kenya, Rwanda and Burundi were estimated at 876,576 tonnes, 615,992 tonnes, 422,590 tonnes and 251,761 tonnes, respectively in 2014 (FAOSTAT, 2014).

In Eastern and Southern Africa, common bean is grown for both subsistence and sale in regional markets, playing an important food security role, since it acts both as a food and income generating crop (Wortmann et al., 2004). The crop is mainly grown for its green leaves and fresh or dry seeds. Dry seeds are, however, the ultimate economic part of the plant (CIAT, 2013). The seeds are mostly eaten whole in cooked dishes typically boiled, often with additives, although they may also be processed to bean flour, purees and spreads (Garden and McNeal, 2013). In tropical Africa common bean may also be mashed or made into soup. In many parts of the world the dry seeds are consumed as canned beans, either alone or in tomato sauce (CIAT, 2013)

Common bean is the second most important source of protein and the third most important source of calories for over 100 million people in rural and poor communities in developing countries (Buruchara, 2007). As such, it has come to be referred to as the “poor man’s meat” for its affordable price; it is also much appreciated by wealthier consumers for its nutritional value (Beebe et al., 2013). The crop, especially acts as a good complement for maize, cassava and rice among other foods due to its richness of lysine that is not present in these crops. It is a rich source of minerals and vitamins, low in fat and cholesterol free; its regular consumption is, hence, highly promoted (Garden and McNeal, 2013).

In Eastern and Southern Africa, levels of consumption of common bean for human nutrition vary with country. In Uganda, consumption of the crop is around 11 kg per person per year while Rwanda has the highest consumption in the world at 29 kg per person per year (Laroche et al., 2014). On average, the crop provides 45% of total protein and 25% of the total dietary calorie intake for the population in Uganda (UNDP, 2012). Although common bean is usually grown for household consumption the surplus is sold to generate income for many, contributing up to 9% of household income in the major producing districts of Uganda (Gatsby, 2014). The level of production varies with region as follows: central (26%), eastern (21%), western (43%) and northern (10%) regions (UNDP, 2012). Production is dominated by small scale farmers, with average plot sizes ranging from 0.1 to 0.5 hectares per household. The average area under common bean production is nearly 920,000 ha, more than 12% of the total cultivated area in Uganda (FAOSTAT, 2014).

Apart from its calorific contribution, common bean also contributes towards alleviation of micronutrient malnutrition (“hidden hunger”) in developing countries, which is common among more than one third of children below the age of five years (Beebe et al., 1999; USAID, 2016). These children suffer deficiencies of Fe and vitamin A, which in severe cases accounts for more than 75% of the deaths of infants and young children (USAID, 2016). These deaths could be significantly reduced with greater consumption of Fe fortified beans although non-fortified beans still provide minerals such as: Fe, Zn, calcium and phosphorous at minimal amounts (Bennink, 2012). In these genotypes (dry beans), Fe and Zn content ranges from 18.8 - 82.4 mg of Fe/kg and from 32.6 - 70.2 mg of Zn/kg (Costa et al., 2006; Mukamuhirwa et al., 2015). Iron is an essential nutrient in human health, preventing anemia and facilitating proper functioning of many metabolic processes. Zinc is essential for adequate growth, sexual maturation and for resistance to gastro-enteric and respiratory infections, especially in children (Bouis, 2003). The crop has numerous health benefits including decreasing the risk of developing obesity, many types of cancer and heart diseases (Garden and McNeal, 2013).

Apart from its relevance in human consumption, common bean is an important source of animal feed. Crop residues are often used as fodder. Dry leaves, threshed pods, and stalks are fed to animals either solely or in combination with supplements (CIAT, 2013). The stems are also used as a source of fuel for cooking, especially in Africa and Asia (CIAT, 2013).

1.1.2 Production constraints of common bean in Uganda

In spite of its importance, however, the full potential of common bean production in Uganda has not been realized due to a number of production constraints. These constraints include biotic and abiotic stresses that account for 52% and 43% of the total grain yield loss, respectively (UNDP, 2012). Diseases are the second biggest constraint to bean production, after low soil fertility, in East Africa (Katungi et al., 2009). The common diseases include: common bacterial blight (*Xanthomonas sp.*), angular leaf spot (*Pseudocercospora griseola*), anthracnose (*Colletotrichum lindemuthianum*), bean root rots (*Pythium sp.*, *Fusarium sp.*, *Sclerotium rolfsii*, and *Rhizoctonia solani*), rust (*Uromyces appendiculatus*) and Bean common mosaic virus disease (Wortmann et al., 2004). The most destructive pests have been identified as; bruchids [the Mexican dry bean weevil *Zabrotes subfasciatus* (Boheman)], and the bean weevil *Acanthoscelides obtectus* (Say), aphids (mainly *Aphis fabae*); pod borers (*Helicoverpa spp.* and *Maruca testulalis*); bean stem maggot (*Ophiomyia spp.*); foliage beetles (*Ootheca spp.*) and thrips (*Megalurothrips spp.*) (Wortmann et al., 2004). Pests are becoming more of a challenge due to climate change. As a result of increased warmer temperatures, new pests are able to invade previously uninhabitable areas and also develop resistance to pesticides (CIAT, 2013). The major abiotic constraints in common bean production include drought stress and low soil fertility (Wortmann et al., 2004; Buruchara, 2007).

1.1.3 Consumer preferred traits in common bean

Consumer preference is increasingly gaining prominence as a determinant of per capita consumption of common bean. This is governed by various factors such as the occupations and settlement area as well as mode of preparation (Muyonga et al, 2008). These preferences range from visual characteristics such as: seed color and shape, to culinary properties like cooking time and texture of cooked beans (Cichy et al., 2012). Short cooking time is widely emerging as a consumer preferred trait for variety acceptability and adoption (Torga et al., 2011). Lately, micro nutrient content is also being emphasized to combat the increase in micronutrient malnutrition. Unfortunately, many high yielding and pest resistant genotypes lack consumer preferred traits such as short cooking time and high micronutrient (Zn and Fe) content (Cichy et al., 2012).

1.2 Problem statement

Over the past 20 years, the Pan-African Bean Research Alliance (PABRA) has released over 550 new bean genotypes across countries in western, southern and eastern Africa (PABRA, 2014). Many of these genotypes are high yielding, with resistance to major pests and diseases; and improved tolerance to abiotic stresses. They are also of various seed sizes, seed color including tans, yellow, white, black and red beans; and possess different culinary qualities (CIAT, 2013). However, there has been limited focus on culinary properties such as cooking time yet the “hard to cook” (HTC) defect is a major consumer acceptability consideration (Muyonga et al., 2008). Prolonged cooking time is especially a concern among urban consumers due to the time invested in cooking and the high cost of fuel energy, in the form of kerosene and gas that are preferred by these consumers (Cichy et al., 2012). Firewood and charcoal form cheaper alternatives for rural and urban dwellers respectively, although at a high cost to the environment (Maryanna et al., 2010).

Prolonged cooking time also leads to structural changes at the grain cellular level, resulting in loss of micronutrients (Ribeiro et al., 2013) such as Fe and Zn, which play important roles in human nutrition. Inadequate micronutrient consumption has resulted in rampant micronutrient malnutrition (USAID, 2016). Several strategies exist for combating micronutrient malnutrition, including supplementation and food fortification (Zulu, 2013). Supplementation is, however, a short term strategy; it is also expensive and has limited coverage. Supplementation is, thus, not a viable option for the rural poor (Bouis, 2003). Breeding for bean genotypes with short-cooking time and enhanced micronutrient content would be cost-effective not only for the rural poor but also for the government that invests resources in treating malnutrition (CIAT, 2013). Long cooking time bean types have been shown to lose more micronutrients during the cooking process (Cichy et al., 2012) compared to shorter cooking time types. However, not much research has been done to identify lines that possess these traits among existing bean germplasm. The mode of inheritance and gene interactions governing the traits in common bean are also not well understood.

Studies by Blair et al. (2005; 2009; 2010) reported inheritance of micronutrient traits to be under the control of multiple genes. Other studies, however, suggest that inheritance of Zn concentration in common bean may be determined by one or two genes (Cichy et al., 2005). Expression of seed cooking time is also reported to be governed by a few genes (Jacinto et al.,

2003) although other studies highlight the contribution of maternal effects against a background of dominant gene control (Elia, 2003). In order to determine the mode of inheritance of these traits, an appropriate mating design has to be applied, using the best possible parents. Combined selection based on grain yield and both cooking and nutritional quality is a new area for common bean, and research is still very much in its infancy (Ribeiro et al., 2013). In order for the National Bean Breeding Program of Uganda to develop genotypes with these traits, this research was required to help understand the genetic factors controlling these traits.

1.3 Justification

Common bean can contribute significantly to end hunger, achieve food security and improved nutrition (PABRA, 2015). The crop is affordable and an important source of protein, fiber, carbohydrates, folic acid, Fe and Zn, tackling micronutrient malnutrition especially among children under five years and expectant mothers (Beebe & Andersson, 2015).

Breeding for short cooking time, high Fe and Zn content in common bean is more feasible to combat the micro nutrient malnutrition (Bouis, 2003). However, this relies on genetic diversity of these traits in common bean to make progress. Cooking time, seed Fe and Zn concentration vary with genotype in common bean (Correa, et al., 2010; Zacharias et al., 2012). This variability enhances the potential for breeding for improved nutritional quality (Zacharias et al., 2012) and short cooking time. Characterization of genotypes for these traits is thus vital for identification of suitable parents to be used in the development of genotypes to meet the specialized micronutrient needs of pregnant women and children while at the same time saving time and fuel energy in preparation of beans.

Knowledge on heritability of short cooking time and high mineral concentration in common bean is important for the development of genotypes with the combination of desired traits (Jacinto et al., 2003). Previous studies have reported cooking time having high narrow sense heritability [0.9 (Elia, 2003), and 0.74 (Jacinto et al., 2003)]. The trait is also governed by multiple genes, with partial dominance of short cooking time over long cooking time (Elia, 2003). The inheritance of nutritional traits appears to be mostly quantitative and only somewhat influenced by the environment, but with variation that is dependent on the source genotype (Cichy et al., 2005). Previous studies by Nchimbi-Msolla and Tryphone (2010) have highlighted the

possibility of selecting for enhanced Fe content in common bean by targeting Zn content. Strong positive correlation between Fe and Zn concentrations ($r = 0.75$) was reported by Mukamuhirwa et al., (2015) suggesting that these micronutrients are not independently inherited. The positive relationship between Fe and Zn seed content plus the negative relationship observed between Zn and seed size ($r = 0.56$) reported by Mukamuhirwa et al. (2015) suggest that Zn and Fe accumulation is controlled multigenically or oligogenically, with some genes affecting the concentration of both minerals. Studies have revealed high genetic effects on cooking time, Fe and Zn content in common bean suggesting the possibility of breeding for these traits. This study established the relationship between cooking time, Fe and Zn content in order to facilitate breeding for simultaneous improvement of these traits.

1.4 Objectives

The aim of this study was to contribute towards the development of fast-cooking and micronutrient -rich common bean genotypes by:

1. Screening of common bean genotypes for short cooking time, high Fe and Zn content
2. Determining the mode of inheritance for cooking time, Fe and Zn content in common bean genotypes

1.5 Hypotheses

The study was based on the premise that:

- 1) Common bean genotypes have great genetic diversity with respect to cooking time, Fe and Zn content.
- 2) Common bean genotypes whose seeds have high water absorption capacities cook much faster than those with seeds that are less hydrated.
- 3) High Iron content in common bean is highly correlated with high Zn content.
- 4) The combination of short cooking time, high Fe and Zn content in common bean genotypes is not associated with type and gene pool.
- 5) Cooking time, Fe and Zn content in common bean are primarily under additive gene control.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin, classification and occurrence Common bean

Common bean (*Phaseolus vulgaris*) has two major gene pools, one from wild beans originating from the region of Northern Mexico to Colombia [(Mesoamerican gene pool (MA))] and the other originating from Southern Peru to Northwest Argentina (Andean gene pool) (Freyre et al., 1996). These genepools have distinct variations. Genotypes in the Andean group are predominantly medium or large seeded while those in the Mesoamerican group are either small or medium seeded. The two gene pools also differ in terms of nutritional quality. Andean beans have higher average seed Fe concentration, but significantly lower seed Zn concentration than MA and introgressed-type beans (crosses between MA and Andean). Introgressed genotypes often have higher disease resistance and yield potential than pure Andean beans. They also have medium-sized seed and quality characteristics of Andean beans, but are often smaller seeded than pure Andean beans (Blair et al., 2010).

From the points of origin, common bean was dispersed to other parts of the world. The Portuguese traders introduced the crop to Africa from the 16th century through Sofala (Mozambique), Zanzibar and Mombasa, from where it was spread to other countries and to higher altitude areas of the interior by slave trading caravans and merchants. Common bean, thus, became well established as a pulse crop in parts of Africa before the colonial era (Freyre et al., 1996). In Africa, the Mesoamerican and Andean gene pools are equally-occurring. However, there are striking differences in occurrence between countries due to farmer selection preferences and availability of germplasm from national programs (Katungi et al., 2009).

Wortmann et al. (2004) classified common bean genotypes into 9 major bean market classes according to color and size: pure large reds, medium and small reds and red mottled, purple, yellow and tans, cream, navy/white and black. Market forces and agro-ecological conditions are the major determinants of spatial distribution of seed types (Broughton et al., 2003). The reds and red mottled beans are the most common types grown in Uganda due to market preferences mainly for their seed color which produces the desired broth. An aggregate area share of about 50 percent for pure reds and red mottled exists in Eastern Africa (Wortmann et al., 2004). However, the current preferred market genotypes are less tolerant to the important biophysical

constraints (drought, poor soils and diseases) and the predicted effects of global warming on the climate in the region could alter the trend in variety distribution (Wortmann et al., 2004; Katungi et al., 2009).

2.2 Morphological diversity in common bean

Morphological traits are important in common bean characterization, conservation and breeding (Okii et al., 2014). A wide diversity exists in Uganda in terms of growth habit, seed shape, size and color (Wortmann et al., 2004). Ideally, diversity is great where beans are produced, marketed and consumed as varietal mixtures majorly due to the growing conditions (Katungi et al., 2009). Common bean shows variation in growth habits from determinate bush to indeterminate extreme climbing types. The bush bean type is preferred to the climbing type because of its low production requirement and convenience for market production (Okii et al., 2014). Bushy types are popular in areas where commercial bean production has gained importance because of their early maturing characteristics and less labor requirements. The climbers pre-dominate in the highland areas which have a high population density and limited land, they are preferred due to their high yielding ability. They are commonly grown in south-western highlands of Uganda (Wortmann et al., 2004; CIAT, 2013). In comparison with the bush bean type, climbing beans are less susceptible to diseases and are more productive.

Seed color and size are important quality parameters for consumers, seed sizes range from the small black type to the large white, brown, red, black or mottled seeds. In Uganda, the major bean types grown include: Calima (red flecked) and reds (large and small) accounting for about 50% and have a high market demand. Others are navy, creams, brown-tan, yellow, purple, white and black beans (Buruchara, 2007). The seed color of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and condensed tannins on the seed while Seed size and weight depend on genetic variations, cultivar and environmental conditions (Reynoso et al., 2006).

2.3 Traits of importance in common bean

The importance of good variety traits in uptake and adoption of improved genotypes is an integral component of the bean breeding process (PABRA, 2014). Pan African Bean Research Alliance research agenda is highly influenced by biophysical constraints and user preferences (PABRA, 2014). These include multi-disease resistance, tolerance to low soil fertility, drought escape to address the problem of early ending rains and the problem of intermittent drought.

With global climatic change threatening to intensify the drought problem in some parts of Africa and the rapid population growth, food and nutritional insecurity in Sub-Saharan Africa may increase. New emphasis is being placed on breeding genotypes for higher nutrition that are adapted to abiotic and biotic stresses (CIAT, 2013). With micronutrient malnutrition becoming a public concern, bio-fortification has been recognized by the nutrition and health sectors as one of the strategies that would contribute to combating malnutrition (Zulu, 2013). Apart from nutrition, variety traits like high yields, early maturity, good taste, stress tolerance, low flatulence and fast cooking are popular among many genotypes, reflecting their importance in variety acceptance by the consumers. Farmers, who also double as consumers of beans, put greater weight on post-harvest traits such as taste and cooking time than they do on production traits such as yield or tolerance to environmental stresses (Katungi et al., 2014). As bean processing gains popularity, the trend in preferences for farmers are expected to change (PABRA, 2014). A study by Katungi et al., (2014) revealed that men are more willing to pay for genotypes with short cooking time than women because the fuel wood for cooking now competes with the alternative use for wood which is controlled by men. Breeding efforts are thus paying close attention to disease resistance, bean size, shape and color, as well as post-harvest attributes such as cooking time; this will help to conserve the environment by saving fuel wood (CIAT, 2013).

2.3.1 Cooking time

Common bean is rich in nutrients and micronutrients, which are made available to the human body after a thermal transformation process, also known as cooking. Cooking is fundamental for bean preparation and consumption, as it increases digestibility, inactivates anti-nutritional factors, increases nutrient biological value and provides the sensorial quality and color

characteristics requisite with consumer demands (Tharanathan and Mahadevamma, 2003; Costa et al., 2006). Cooking is probably the most energy-demanding process in the bean value chain. In the USA, industrial processing and home cooking consumes about 48% of the energy in the food chain as compared to 21% and 13% energy used in production and transportation respectively (CSS, 2011). This relatively high energy requirement is due to prolonged cooking time in beans. Common bean can take up to 3 hours to cook, though there is a wide variability ranging from less than 45 minutes (short cooking time), to more than 60 minutes (long cooking time) (Muyonga, et al., 2008). In most developing world, short cooking time is emerging as a consumer-preferred trait (Beebe et al., 2013). Unlike commercial producers who are interested in high yielding and stress tolerant genotypes, subsistence farmers have higher preference for post-harvest traits such as taste and cooking time (Katungi et al., 2014).

Worldwide more sophisticated consumers are ready and willing to pay for an excellent product according to their preferences. In Latin America, color preferences are still paramount. Local producers grow beans of the area's preferred color, which they can sell at high prices. In Africa where mixed genotypes are preferred, uniformity of cooking is a more important factor to the consumers than bean color (Cichy et al., 2012).

Cooking time differs regionally and can be a measure for consumer acceptance. It is of less importance where pressure cookers are used majorly in many Latin American regions as opposed to where firewood is the main fuel source in Sub Saharan Africa (Broughton et al., 2003). Producers are concerned about risk avoidance and yield of good quality beans. They recognize the importance of good adaptation of cultivars and resistance or tolerance to major negative characteristics. They also emphasize on good culinary quality, taste and selected traits such as seed size, color and plant growth habit (CIAT, 2013).

There is a positive correlation between cooking time and protein in cooked beans (Akinyele et al., 1986). Prolonged cooking time destroys the heat labile vitamins and increases the percentage of leached solid; therefore, fast cooking genotypes have a higher nutrient retention by reducing the amount of leached solids, as well as increasing consumer acceptability (Garden and McNeal, 2013).

2.3.2 Factors influencing cooking time

Common bean cooking time is affected by many factors including; seed size, storage time, humidity and temperature of storage environment (Arruda et al., 2012). Long storage time results in changes in bean taste, color and broth, thus decreases the commercial value of the bean seeds (Correa, et al., 2010). Bean storage under high temperature and high humidity lead to the development of the hard- to-cook phenomenon that increases cooking time (Arruda et al., 2012).

Seed characteristics play a role in determining cooking time; these include, grain thickness and flatness (Guilherme et al., 2016). Environment has an effect on cooking time and hydration capacity of beans. It is possible that technological characteristics of the common beans are interconnected with the weather conditions at the seed development and in the pre- harvest period Arruda et al., (2012). To identify genotypes with short cooking time, multi-locational evaluations are required (Zilio et al, 2014).

In cooking time studies, soaking beans prior to cooking is considered as a pre-cooking treatment for reducing the cooking time. High water absorption rate is associated with faster cooking time (Elia, 2003). Previous studies (Elia, 2003; Correa et al., 2010) have indicated an indirect association between cooking time and water absorption. This indicates that, the amount of water absorbed by beans may be used as an indirect method for screening genotypes for cooking time. However, a study by Carbonell, (2003) found a low correlation between water absorption and cooking time.

2.3.3 Genetics of cooking time

A study by USDA (2014) revealed a diversity in cooking time for different bean market classes. Diversity analysis is useful in understanding the genetic control of cooking time and facilitates breeding for fast cooking beans in diverse Andean market classes. Heritability of this trait would then have to be understood for progress in breeding work. Study by Elia (2003) reported heritability for cooking time at 0.9, while that by Jacinto et al., (2003) reported heritability estimate of 0.74. Andean dry beans showed cooking time is governed by genes with partial dominance of short cooking time over long cooking time, with cytoplasmic influences on the expression of short cooking time (Elia, 2003). Short cooking time is also observed to be dominant over long cooking time in cowpea, governed by two dominant alleles interacting at

different loci (Mashi, 2006). Additionally, the genes controlling short cooking time and long cooking time are allelic and all nuclear and cytoplasmic genes have no effect on cooking time. Cooking time has a large genotype effect and high heritability which aids selection based on the trait itself (Elia, 2003). However, this information in beans is scanty.

2.4 Importance of Iron and Zinc content in common bean

Micronutrient deficiency affects more than 3 billion people worldwide (USAID, 2016). Among the deficient micronutrients, Zn and Fe stand out. Zinc plays an important role in the human body, exercising important structural, enzymatic and regulatory functions in living cells (Akond et al., 2011). Zinc deficiency in the human body may cause delays in growth and sexual maturity, alopecia, skin rashes, slow healing of wounds, immunodeficiency, behavioral disorders, night blindness and loss of appetite (Akond et al., 2011). In general, 1.4% of deaths due to “hidden hunger” are attributed to a lack of Zn (USAID, 2016). Iron is essential for preventing anemia and is active in many metabolic processes in the human body, deficiency of this mineral is a serious public health problem that affects millions of people worldwide. One and a half percent (1½%) of the deaths occurring in the planet are attributed to Fe deficiency (Ugen et al., 2014).

Foods that may overcome these nutritional deficiencies in populations are the subject of various studies (Akond et al., 2011; Zacharias et al., 2012). However, these foods, in addition to high nutritional quality must have good commercial and agronomic quality, besides being low cost. In this respect, one of the main options is common bean. This legume is the main nutritional component of the diet for more than 300 million people and has high nutritional and functional value (Beebe et al., 2013).

Dry beans may also benefit people with diabetes since they provide complex carbohydrate and are low in fat. They also supply vital nutrients to the diet, they are low in sodium and contains no cholesterol (Garden and McNeal, 2013). Nutritional quality in common bean has been found to be of great importance; there are large amounts of minerals and vitamins provided by the seed together with a background of high percentage protein, complex carbohydrates and low oil content (Garden and McNeal, 2013).

2.4.1 Potential for improvement for Iron and Zinc content in common bean

Common bean has a higher concentration of bioavailable Fe and Zn, which are retained through harvest and processing unlike most cereal grains (Bouis, 2003; Bouis and Welch, 2010). Increasing Fe and Zn content in common bean may contribute significantly to improving the health status of individuals dependent on beans as a staple food. Significant increases in the amount of bioavailable Zn and Fe in beans can be made using conventional plant breeding techniques (Nchimbi-Msolla and Tryphone, 2010) based on the existing wide variation in concentrations of the micronutrients among bean genotypes.

In terms of biofortification, improvement of mineral content of this crop is feasible because the baseline grain Fe content is high at 55 ppm (mg/kg) and variability for the trait is great, being up to 110 ppm, allowing initial breeding attempts to be much more successful than in cereals in overall Fe and Zn content improvement (Beebe et al., 1999). High Fe concentrations and wide genetic variability have made it possible for plant breeders to develop high Fe bean genotypes (up to 10 mg/100 g) (Zulu, 2013). Bean genotypes with seed Fe content of > 70 mg/kg are considered high in Fe high Zn content is >30mg/kg. The challenge to address is, however, bioavailability of Fe to the human body, hence the target levels of >94mg/kg for Fe and >47mg/kg for seed Zn (Zulu, 2013). Polyphenol content is the major determinant of Fe bioavailability in common bean, which in turn can be indirectly screened for by seed coat color (Hendrich et al., 2013).

2.4.2 Genetics of Iron and Zinc content

Information on genetic control of Fe and Zn content is important when beginning a breeding program aiming to obtain cultivars with higher nutritional quality (Camila et al., 2013). There is large genetic variability in seed mineral concentration for Fe (47 - 77 ppm) than for Zn (28 - 38 ppm) (Mukamuhirwa et al., 2015) and both minerals have cytoplasmic inheritance and a positive relation indicating they are not independently inherited. Studies have reported that the inheritance of Fe and Zn is quantitative (Blair et al., 2009). However, some studies report monogenic inheritance for Zn (Cichy et al., 2005), which varies depending on the source genotype e.g. Mesoamerican beans having lower concentrations of Fe than Andean beans, but higher Zn levels (Islam, 2002). Micronutrient concentration can be improved in bean genotypes

through breeding (Cichy et al., 2005; Blair et al., 2009). It is the most feasible approach due to the low cost and wide consumption of beans. Studies have also reported higher Fe concentration in the climbing beans as opposed to the bush beans (Mukamuhirwa et al., 2015; Blair et al., 2010). This is associated with the longer days in the field by the climber beans hence the ability to take up more minerals from the soil (Camila et al., 2013).

In 2010, Nchimbi-Msolla and Tryphone reported that both genotypes and environment influence the concentration of iron and zinc in seeds of bean and that the superiority of a genotype in iron and zinc is conditioned by the environment and to the genotype by environment interactions which influences the selection of elite cultivars adapted to wide regions.

2.5 Profile of common bean genotypes in Uganda

The major variations in common bean germplasm in Uganda are attributed to growth habit, pod cross-section, pod curvature, hypocotyl color, days to flowering, number of flower buds and 100-seed weight (Okii et al., 2014). There is a large phenotypic diversity among preferred common bean genotypes due to varietal mixtures grown by the farmers and consumers tend to accept a wide range of seed colors (Blair et al., 2010). Farmers use varietal mixtures as a mechanism of coping with environmental variability and to diversify production in small plots. Early and late maturing components, thus, together provide harvestable products over a long period. However, this diversity is under threat due to economic and agronomic developments that have shifted emphasis to single-component (trait) genotypes over multi-component mixtures. This is mainly due to consumer demand for pure lines which are easy to prepare due to their uniformity as opposed to mixtures (Blair et al., 2010).

Apart from phenotypic variations, local and market preferences as well as the variability in climatic and agronomic conditions generally dictate which genotypes are most popular (Broughton et al., 2003). Farmers in Uganda majorly grow the bush bean type which are not as rich in Fe and Zn content as climber beans though have the potential for improvement given the wide micronutrient content diversity. Large red-mottled genotypes form the big part of most popular genotypes in Uganda. They comprise of some of the traditional types such as K20, a determinate variety developed by the National Bean Research Programme in the 1960's (Katungi et al., 2009) and "*Nambale*" a semi climber large red mottled bean. Other important local types

available in the country include “*Kayinja*” a medium size type, “*Kanyebwa*” the red-medium type and the brown-red oval types. The small-seeded “*Lango*” beans are usually black or cream colored bush bean genotypes and are popular in Northern Uganda. However, some of the new improved genotypes developed by the National Agricultural Research Organization (NARO) and other partners have also received high market reception especially K132 a Calima seed type, a determinate bush type with dark red mottled large seeds, K131 a Carioca seed type, indeterminate bush type with small beige seeds and NABE 2 a small black seed type (PABRA, 2014). Several other bean seed types are cultivated in Uganda, with definite regional differences in preferences for production and consumption, including black beans (mostly in northern Uganda) and white beans (UNDP, 2012).

Over a period of 5 years (2009-2014), 172 multiple stress resistant varieties have been released in eastern and southern Africa. They combine resistance genes to major diseases (Bean common mosaic virus, anthracnose, angular leaf spot, common bacterial blight and root rots), low soil fertility tolerance, drought tolerance and to some extent high Fe and Zn content (PABRA, 2014). Breeding programmes have made progress in development of improved bean varieties with focus on improved high yield, robust, high-iron varieties for a wider range of agro-ecological zones, covering a broad range of market classes (grain color and size, cooking time, and taste) (PABRA, 2014). Some of the released genotypes for multiple stress resistance and high Fe and Zn content include; RWR2245, RWR2154, RWR1129, MAC44, VCB81013, RWV3006, RWV3316, RWV3317 and MAC42 (Beebe and Andersson, 2015).

CHAPTER 3

IDENTIFICATION OF COMMON BEAN GENOTYPES WITH SHORT COOKING TIME, HIGH IRON AND ZINC CONTENT

3.1 Background

Cooking time is currently a priority area for common bean improvement due to its implications for energy utilization, nutritional value and gender equity (USDA, 2014). Breeding programs are focused on the identification and development of fast-cooking bean genotypes to increase consumption and market acceptability. The market prefers genotypes that can be cooked for less than one hour because of lower utilization of energy and time for meal preparation. In addition, fast-cooking bean genotypes retain more nutrients than longer cooking beans (Cichy et al., 2014). Cooking times for beans can vary from 1 ½ to 3 hours, depending on variety and cooking method used (Elia, 2003). The scarcity of firewood in Eastern Africa has made reduction in resources required to prepare beans for eating an important economic consideration (Mashi, 2006). Fast cooking bean cultivars would be a means to attain food security while conserving firewood and providing adequate nutrition for consumers (Petry et al., 2015). In addition, prolonged cooking time results to higher loss of starch, protein and Iron (Cichy et al., 2013)

In Uganda, persistent under nutrition in children is a perilous issue given that 33% of children under five years are stunted and 14 % are underweight. In addition, under nutrition is a core cause of 60 % of deaths for children under five years (USAID, 2016). Micronutrient deficiencies are highly prevalent in women and children (USAID, 2016) and result in an enormous negative socio-economic impact at individual, community and national levels (CIAT, 2013). Undernourished populations lack the energy required for agricultural production, which is the country's backbone. There is a heavy burden of care imposed on the mothers of undernourished children. Government spending in the fight against malnutrition also hinders more development programs. To combat micronutrient malnutrition, research plays a key role in scaling up the production and marketing of bio-fortified varieties like orange-fleshed sweet potato rich in Vitamin A and beans bio fortified with Zn and Fe (USAID, 2016).

Availability of bean genotypes with high seed Fe and Zn within farming systems will go a long way towards combating nutritional disorders associated with these nutrients (Buruchara et al., 2011). The starting point is to determine the seed Fe and Zn content in existing bean germplasm

and promote the consumption of such bean types or their utilization in breeding for improved mineral content. In addition, germplasm with high seed Fe and Zn content should not be exploited unless they withstand other production constraints in the region. Some of these are diseases such as angular leaf spot (ALS), anthracnose, ascochyta blight, Bean common mosaic virus (BCMV), Bean common mosaic necrotic virus (BCMNV), common bacterial blight (CBB), rust (PABRA, 2014).

Breeding progress for improving any trait is proportionate with the amount of genetic variability in the population. Evaluating genetic variability for a trait needs screening large amounts of germplasm. This study was conducted to establish the cooking time and micronutrient (Fe and Zn) content in bean seed grown and consumed in Uganda.

3.2 Materials and methods

3.2.1 Study site

The study was conducted at the International Centre for Tropical Agriculture (CIAT) Uganda based at the National Agricultural Research Laboratories (NARL) in Kawanda. The station is located in Wakiso district in central Uganda, at Latitude 0° 23' 39" North, Longitude 32° 32' 11" East. It stands at an elevation of 1193 m above sea level, with the mean annual rainfall of 1250 mm, daily temperatures average 15.3°C minimum and 27.3°C maximum, relative humidity of 76.3% and soil that is of a sandy loam type with pH of 5.5-6.0 (Fallingrain, 2015).

3.2.2 Genotypes used in the study

One hundred and fifty two (152) common bean genotypes were evaluated; 121 bush beans and 31 climbers. This germplasm consisted of released bean (commercial) lines from seven East African countries namely; Ethiopia, Madagascar, Uganda, Kenya, Tanzania, Rwanda and Burundi (PABRA, 2015). Breeding parents for important traits (Table 1) commonly used by members of the Pan-Africa Bean Research Alliance (PABRA) being maintained in the regional common bean gene bank at CIAT-Uganda were used.

Table 1 : Common bean germplasm used in the study and their characteristics

Origin	Official Name	Desired trait
BURUNDI	AND10, BIHOGO, G685, MSO'LE, MUKUNGUNGU, VCB81013, GASIRIDA, GLP2, HM21-7, MAC 44	Drought tolerant High Fe and Zn content, high yield,
D.R. CONGO	ACC714, AFR708, AND10 CAL143, CNF5520 G685, G2858, G2333, JESCA MAHARAGI SOYA, CODMLB001, G858, GLP2, MLB49-89A, M'SOLE, NAKAJA NGWAKU-NGWAKU, ROBA-1, NUA45, ZEBRA, HM21-7, RANJONOB	High Fe and Zn content, pest and disease resistant, high yield, adaptation, short maturity time, market type, low soil fertility tolerance
ETHIOPIA	AWASH 1, AWASH MELKA, ROBA-1	Preferred Market type, drought tolerant
MADAGASCAR	RANJONOMBY,	Disease resistant
KENYA	G2333, G685, GLP2, KATB1, KATB9, KATX69, KATX56, KK20, KK8, MLB-49-89A, RWR1092, SCAM-80CM/15, ZEBRA	Pest and disease resistant, high yield, short maturity time, preferred market type
RWANDA	G2333, GASIRIDA, MAC42, MAC44, MLB49-89A, RWR2154, RWR2245, RWV2887, RWV3006, RWV3316, RWV1129	High Fe and Zn content, and market type, yield, drought tolerant
TANZANIA	A197 (S.TZ), JESCA (N.TZ), ROBA-1	High yield, fast cooking and drought tolerant
UGANDA	CAL96, K131, K132, NABE1, NABE10C, NABE12C, NABE13, NABE14, NABE16, NABE17, NABE18, NABE19, NABE2, NABE21, NABE22, NABE23, NABE3, NABE4, NABE5, NABE7C, NABE8C, NABE9C, NABE26C	Preferred Market type, high yield, pest and disease, drought tolerant

*Source: PABRA database, 2015. Database.pabra-africa.orgS.TZ= South Tanzania, N.TZ = North Tanzania

3.2.3 Experimental design and management

Two separate trials were set up: one for the bush beans and another for the climbers for ease of management practices and to reduce inter-plot interference likely to occur if planted in one trial. The trials were set up during the 2015B season (April to July) and 2015D (September-December), using a 6 x 21 alpha lattice design with three replications for bush beans and a 3 x 9 alpha lattice with three replications for climber beans. Fertilizer (NPK 17:17:17) was applied during planting at the rate of 100 kg/ha (a recommendation for the experimental site used). Weed management was done using a selective herbicide beans clean (Clethodim 240g/L) at three weeks after germination followed by manual weeding two weeks later. Routine spraying against pests and diseases was done weekly from three weeks after planting to when the beans reached physiological maturity, using fungicide Ridomil at 2.5kg / ha (mancozeb and metalaxyl-M) for control of infections from oomycetes and insecticide Rocket (Cypermethrin 50g/l).

The genotypes were evaluated alongside six Fe seed content checks. Climbers were evaluated alongside MIB 456= universal high Fe, RWV1129= regional high Fe (East Africa) and Decelaya = universal low Fe while for Bush beans, RWR2154= regional high Fe, DOR 500= universal low Fe and CAL 96= regional low Fe were used.

3.3 Data collection

Soil analysis was done for the trial site to ascertain the soil mineral concentration. Data collection started a month after planting. Data were collected weekly on agronomic traits such as growth habit, flower color, days to flowering, days to physiological maturity, plant vigor, pods per plant and the seeds per plot based on the common bean crop ontology trait dictionary. Disease severity data collected included anthracnose, common bacterial blight, angular leaf spot, BCMNV, and rust using the CIAT disease scale (Schoonhoven et al., 1987). No data were collected on insects damage because there were no key field pests noted. Yield data was collected on plot basis by estimating the total (total sample weight per plot before cleaning and sorting) and clean yield (after cleaning and sorting) after harvest. Cooking time and Fe and Zn data was collected on plot basis two weeks after harvest and sun drying of the seeds to 12 - 13 % moisture content determined using a moisture meter.

3.3.1 Soil sampling and analysis

Soil samples were obtained from the trial site by random sampling a week after field establishment. Soil samples were obtained at 0-20 cm depth and away (0.5 meters) from the planted rows to avoid effect of applied fertilizer. The samples were analyzed for, pH, exchangeable magnesium (Mg), calcium (Ca), potassium (K), available phosphorus (P), total organic carbon, and total nitrogen (N); at Kawanda Agricultural Research Laboratories (KARL) following the Soils and Soil Fertility Management Programme protocol of National Agricultural Research Laboratory, Kawanda. The soil pH was determined using the H1 9017 Microprocessor pH meter in 1:2.5 suspension of soil and water. Exchangeable bases (Ca, Mg, K and Na) in the soil were determined in 1.0 M ammonium acetate extract (Okalebo et al., 2002) by flame photometry (K^+ , Na^+) and atomic absorption spectrophotometry (Ca^{2+} , Mg^{2+}). Available P was extracted using the Mehlich-3 extraction method with pH 2.5. Soil organic matter was analyzed using the Walkley Black method, nitrogen was analyzed using sulphuric/selenium digestion mixture, digested at 330°C and later quantified calorimetrically using the Nesler method. For Fe (Fe^{2+}) and Zn determination, undiluted sample extracts were directly aspirated into the atomic absorption spectrophotometer (SHIMADZU AA-6800) and read at 248.3nm and 213.86nm (Gerwing and Gelderman, 2005).

3.3.2 Agronomic traits

Data on plant vigor were evaluated visually at flowering (when the plants reach their maximum development). The scale used was 1 to 9, where, 1 = excellent, 3 = good, 5 = intermediate, 7 = poor and 9 = very poor (Schoonhoven et al., 1987). Days to flowering were evaluated visually by counting the number of days from planting to the day when 50% of plants had at least one flower, with observations made weekly (Schoonhoven et al., 1987). Morphological traits including growth habit, seed color and seed size were also recorded. Growth habit was evaluated at flowering and pod formation using 5 categories named from 1-5 where 1 = determinate bush, 2 = indeterminate bush habit, erect stems and branches, 3 = indeterminate bush habit with weak main stem and prostrate stem and branches, 4 = indeterminate climber habit with weak, long and twisted stem and branches, 5 = determinate climber (Schoonhoven et al., 1987). Data on primary and secondary seed color, seed size and 100-seed weight were scored after harvest. Primary seed

color was evaluated visually using the 1 to 9 scale for the predominant seed color where 1 = white; 2 = cream-beige; 3 = yellow; 4 = brown-maroon; 5 = pink; 6 = red; 7 = purple; 8 = black; 9 = others (Schoonhoven et al., 1987). Seed size was evaluated by weighing 100 seeds on an analytical scale in grams where small size: < 25g/100 seed, medium size = 25-40g/100 seed; large size >40g/100 seed.

3.3.3 Yield data

Yield data was collected at physiological maturity (when pods lose their pigmentation and begin to dry). This was obtained by estimating the pods per plant (5 plants per plot selected randomly) and the seeds per pod (5 pods per plant selected randomly). In the present study, a replication for one trial had been harvested before yield data collection due to some genotypes attaining physiological maturity earlier and resulting to pod shattering hence the data was estimated after harvest. Yield was estimated in grams per plot by the difference in the total yield per plot and the clean yield per plot after harvest and threshing (Schoonhoven et al., 1987).

3.3.4 Disease data

Disease severity data was recorded weekly between the flowering stage (opening of first flower) through pod formation to pod filling (when the first pod begins to fill up to initiation of defoliation) (Schoonhoven et al., 1987). Data on the key common bean diseases under field conditions were recorded, including resistance to angular leaf spot (ALS), Bean common mosaic virus (BCMV), Bean common mosaic necrotic virus (BCMNV), common bacterial blight (CBB), rust, and anthracnose were recorded under field conditions using the new trait dictionary (Schoonhoven et al., 1987; CIAT, 2013).

Angular leaf spot in the field was evaluated by quantifying symptom development using the scale of 1 to 9 where 1 = no visible disease symptoms, 2 = unspecified intermediate values that correspond to intermediate percentages of affected areas, 3 = presence of a few small non-sporulating lesions that cover approximately 2% of the leaf surface area; 4 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 5 = presence of several small lesions with limited sporulation that cover approximately 5% of the leaf surface

area; 6 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 7 = abundant and generally large sporulating lesions that cover approximately 10% of the leaf surface area. On the foliage the lesions may coalesce to produce larger infected areas associated with chlorotic tissue. Lesions may also be found on the stem and branches; 8 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 9 = 25% or more of the leaf surface area is covered by large sporulating and often coalescing lesions (Schoonhoven et al., 1987).

Bean common mosaic virus was evaluated by quantifying symptoms (irregular shape of leaves, malformation of leaves, down curling of leaves, light yellow and dark green area in a mosaic pattern) using 1-9 scale where 1 = no visible symptoms, 2 = doubtful symptoms, 3 = very light symptoms resulting in little or no economic damage, 4 = moderate symptoms, 5 = moderate symptoms, 6 = visible and conspicuous symptoms resulting in only limited economic damage, 7 = presence of general symptoms, 8 = intense infection, 9 = severe symptoms causing considerable yield loss or plant death. Bean common mosaic necrotic virus was evaluated in percentage by counting the number of plants with systemic vein necrosis in leaves, and pods or localized necrotic leaf lesions per total number of plants in the plot (Schoonhoven et al., 1987).

Common bacterial blight on leaves was evaluated by quantifying symptom development using a 1 to 9 scale where 1 = no visible disease symptoms; 2 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 3 = presence of a few small lesions that cover approximately 2% of the leaf surface area; 4 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 5 = approximately 5% of the leaf surface area covered by small lesions that are beginning to coalesce and sometimes encircled by yellow halos resulting in minor blight; 6 = unspecified intermediate values correspond to intermediate percentages of affected areas; 7 = approximately 10% of the leaf surface area covered with medium and large lesions which are usually accompanied by yellow halos and necrosis; 8 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 9 = 25% or more of the leaf surface area is covered by large coalescing and generally necrotic lesions resulting in the defoliation.

Rust on leaves was evaluated by quantifying symptom development at flowering using a 1 to 9 scale, where 1 = highly resistant: no visible rust pustule present (immune), 2 = unspecified

intermediate values that correspond to intermediate percentages of affected areas; 3 = resistant: presence of only a few and generally small pustules on most plants that cover approximately 2% of the foliar area; 4 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 5 = intermediate, presence of generally small or intermediate pustules on all plants that cover approximately 5% of the foliar area; 6 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 7 = susceptible: presence of mostly large pustules often surrounded by chlorotic halos that cover approximately 10% of the foliar area; 8 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 9 = highly susceptible: presence of large and very large pustules with chlorotic halos, that cover more than 25% of the foliar tissue and cause premature defoliation (Schoonhoven et al., 1987).

Anthrachnose on pods was evaluated by quantifying symptoms using 1 to 9 scale where 1 = no visible disease symptoms, 2 = unspecified intermediate values that correspond to intermediate percentages of affected areas, 3 = presence of very few and small lesions that cover approximately 1% of the pod surface area, 4 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 5 = presence of several small round lesions (less than 2 mm in diameter), with sporulation that cover approximately 5% of the pod surface area; 6 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 7 = presence of medium-sized (larger than 2 mm in diameter) lesions are evident but also some small and large lesions generally with sporulation and that cover approximately between 10% and 20% of the pod surface area; 8 = unspecified intermediate values that correspond to intermediate percentages of affected areas; 9 = presence of numerous, large, sporulating, sunken cankers that cover approximately 50% or more of the pod surface area and that can result in pod malformation, low seed number, and death of the pod (Schoonhoven et al., 1987).

3.3.5 Cooking time estimation

Seed samples for cooking time estimation were obtained on plot basis, thus every entry was replicated thrice. After harvest and cleaning, the seeds were sun-dried to 12-13% moisture content and stored in paper envelopes for two weeks at room temperature. Twenty five seeds per plot were randomly picked and weighed using a top-pan balance to obtain weight before soaking. The seeds were then soaked for twelve hours in distilled water and re-weighed to obtain the weight after soak. The amount of water absorbed was scored by obtaining the difference in weight before and after soak and expressed as a percentage (Elia, 2003). Thereafter the seeds were placed in each of the 25 cylindrical holes of the Automated Matson Bean cooker developed by Canadian Grain Commission (Winnipeg, Canada) (Proctor and Watts, 1987) using a burner set at 350 °C and cooking timing started (Wang and Daun, 2005). A Matson cooker is a stand-alone machine monitored by a computer and the test results are automatically recorded on the computer (Plate 1). Cooking time was calculated when 80% of the beans are soft enough to be pierced through by the pin, this is an equivalent of when the 20th of the 25 pins of the cooker has penetrated the seed (Wang and Daun, 2005).

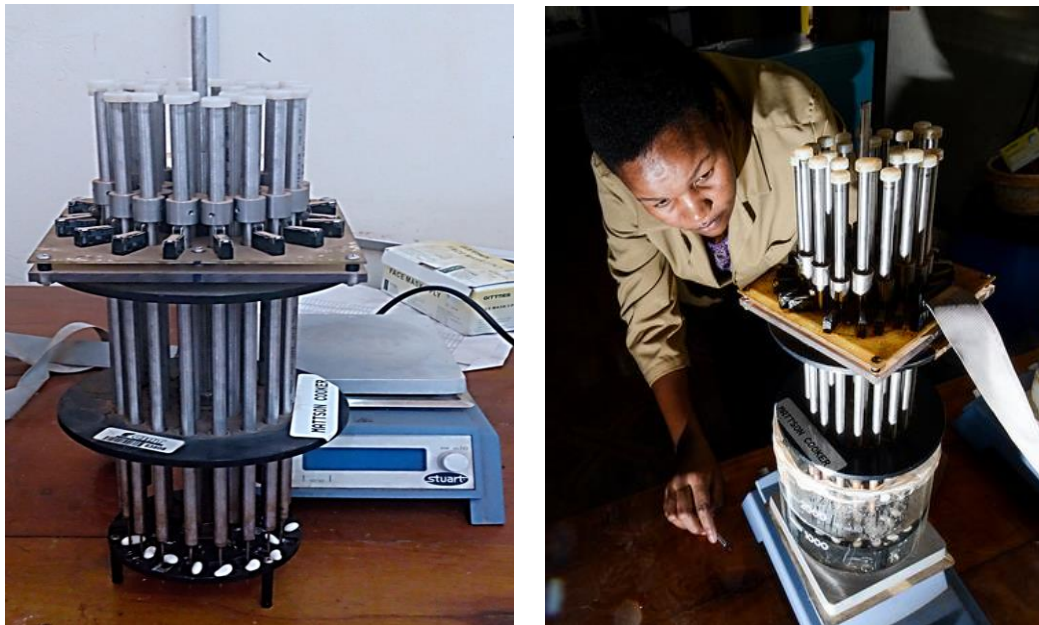


Plate 1: Bean cooking time estimation using the Mattson cooker

3.3.6 Seed Iron and Zinc analysis

After harvesting, threshing, cleaning of seeds, sun drying was done for a period of two weeks to achieve a 12-13% moisture content and bulking per plot done. A sample of 45-50 seeds was randomly picked from each plot cleaned with cotton cloths dampened with distilled water for 60 seconds in order to reduce possible Aluminum and Fe contamination (Paltridge et al., 2011). Samples were then oven-dried at 60°C for at least 12 hours, and then ground using a Sunbeam Conical Burr Mill EM0480 Grinder (Sunbeam, Australia) at a coarse (20-25) setting and subsequently a finer (0-5) setting. Ground samples were stored in paper bags for XRF analysis at the Rwanda Agricultural Board - Rubona station. The grinder was cleaned between samples using a brush and vacuum (Stangoulis, 2010).

Ground samples were transferred into sample cups in the X-ray fluorescence (XRF) machine (Acute instruments, Mumbai, India) (Plate 2). The amounts of Fe and Zn were determined by spectrophotometry with each sample being scanned for 100 seconds. (Oxford Instruments, 2009).

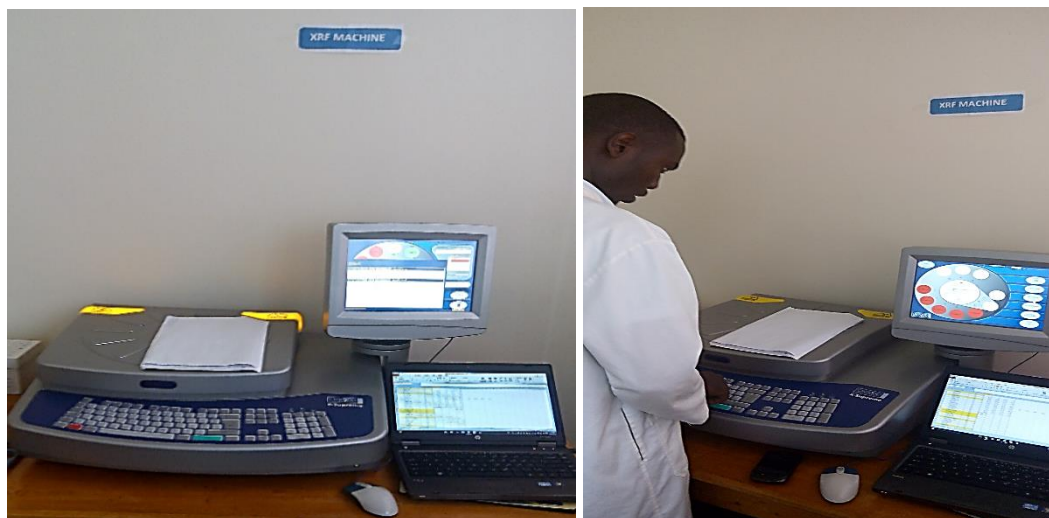


Plate 2: Seed Iron and Zinc analysis in bean seeds using the XRF machine

3.4 Data analysis

Genotype effects for all data including agronomic data, disease data, cooking time, Fe and Zn were subjected to analysis of variance (ANOVA) statistical procedure using Gen Stat software (12th Edition, VSN International Ltd. Copyright 2009). Correlation analysis was done using Genstat.

3.5 Results

3.5.1 Soil status of experimental site

The experimental soils had acidic soils, low soil phosphorus 7.7-7.9 ppm (though not critical) and low K ppm during both seasons. The concentration of minerals such as Ca, Mg and Zn were not sufficient. Organic matter (6.5%), nitrogen (0.31%) and Fe (259.0ppm) concentrations were high to very high and sufficient. The sites did not differ significantly in terms of the soil properties as shown in Table 2.

Table 2: Soil Status of trial site for evaluation of common bean genotypes at Kawanda

Soil component	2015B Measurements	2015D Measurements	Critical values	Sufficient levels for beans
pH	4.95	4.2	5.2	5.2-7.0
OM (%)	6.5	5.7	0.20	0.30
N (%)	0.31	0.38	3.0	6.0
P (ppm)	7.9	7.7	5.0	20.0
Ca (ppm)	1488	1598.77	150.0	500
Mg (ppm)	408.8	389.64	100.0	600.0
K (ppm)	35.11	339.64	350.0	2000.0
Fe (ppm)	259.9	161.0	5.5	50
Zn (ppm)	1.65	5.4	4.0	20

*ppm-parts per million

3.5.2 Variability in cooking time of the genotypes evaluated

The analysis of variance revealed strong significant differences ($P < 0.001$) among the genotypes, seasons and the interaction between genotype and season for cooking time (Table 3).

Table 3: Mean square values for cooking time of bean genotypes evaluated at Kawanda

	Bush		Climbers	
SOV	DF	CT	DF	CT
Season	1	99126*	1	665
Rep/Season	4	2522***	4	665*
Genotype	120	1075***	30	674*
Genotype. Season	120	462***	30	217
SED		9		9
%CV		19.6		16

Ns=non-significant, ** =significant at $P=0.01$ and ***= significant at $P=0.001$, SOV= source of variation, DF = degrees of freedom, CT = cooking time, CV = coefficient of variation, SED=standard error of difference

There was a wide variation in cooking time among the 152 test genotypes in both seasons. For the first season (2015B) a normal rainy season with an average rainfall of 690 mm, cooking time ranged between 35 (Awash melka) and 100 minutes (VAX4) with an average of 56 minutes. Twenty four percent (24%) of the genotypes had cooking time of less than 45 (<45) minutes, 49% with 46-60 minutes and 27% with >60 minutes.

In the second season (2015D), a normal rainy season with an average of 489 mm, the cooking time among the 152 genotypes ranged between 43 (CNF5520) to 122 minutes (RWR2154) with an average time of 73 minutes. This represented 1% (<45 minutes), 24% (46-60 minutes) and 74% (>60 minutes). Awash melka and VAX4 cooked for 51 and 114 minutes, respectively.

Across the two seasons, the average cooking time for the genotypes was 66 minutes, with 3% (<45 minutes), 36% (46-60 minutes) and 61% (>60 minutes) (Figure 1). Several genotypes showed consistency in short cooking time (< 60 minutes) across the two seasons with others cooking for > 60 minutes (Table 4).

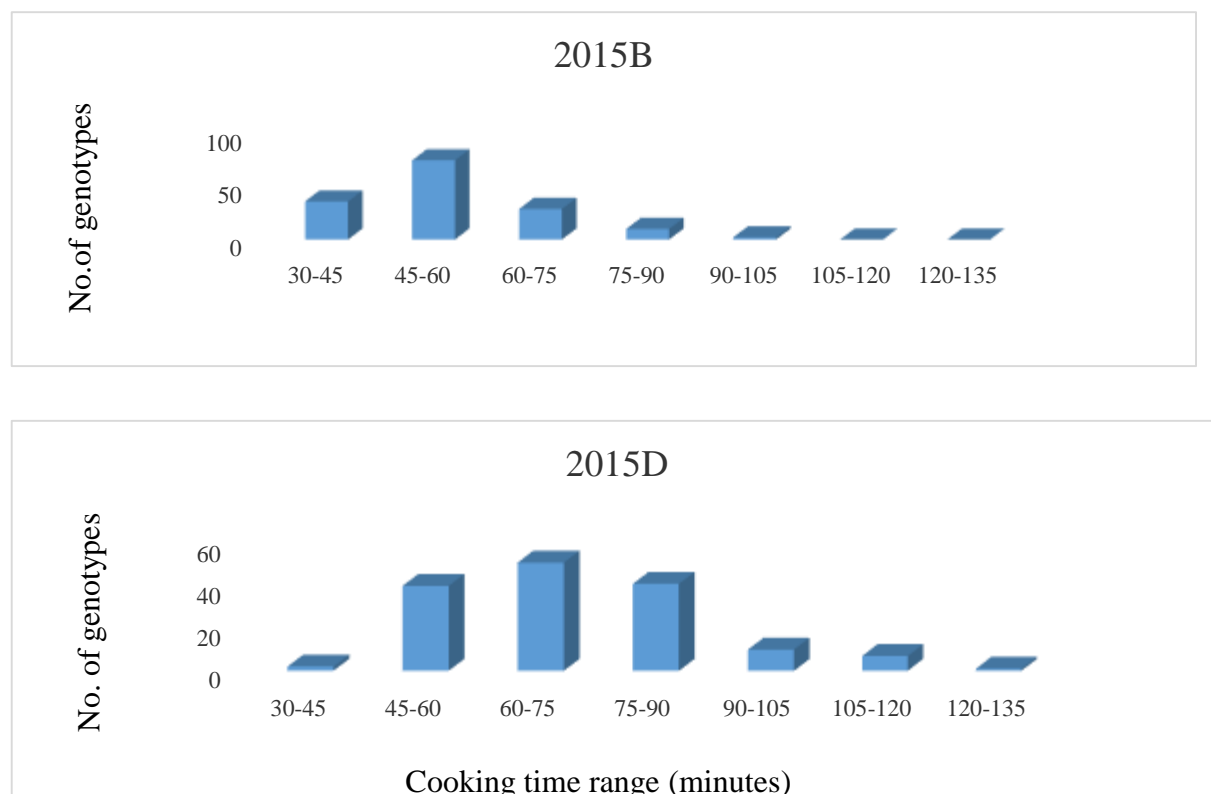


Figure 1: Range of cooking time for 152 bean genotypes evaluated over the two seasons at Kawanda

Table 4: Seasonal performance of genotypes for cooking time

GENOTYPE	Season 2015B		Season 2015D		Average	
	CT (mins)	Rank	CT (mins)	Rank	CT (mins)	Rank
< 60 minutes						
Amahunja	42	6	52	6	47	6
Awash melka	35	1	51	5	43	2
Bihogo	45	9	53	7	49	8
CAB 2	45	9	41	1	43	2
CNF5520	41	5	43	2	42	1
ECAPAN021	47	11	59	13	53	12
G858	44	8	58	12	51	10
Icaquimbaya	46	10	57	11	52	11
KK20	50	14	57	11	53	12
NABE12C	42	6	60	14	51	10
NABE4	45	9	59	13	52	11
NABE6	42	6	49	4	45	5
ROBA-1	44	8	60	14	52	11
RWR 1873	38	3	58	12	48	7
RWV3006	46	10	54	8	50	9
> 60 minutes						
VAX4	100	47	108	53	104	55
KATX56	92	46	106	52	99	53
Kanyebwa	84	42	98	47	91	49
NABE8C	71	32	110	55	91	49
NABE3	71	32	109	54	90	48
RWR 1092	75	36	105	51	90	48
TO	83	41	94	44	88	47
AND 1062	68	30	115	59	92	50
KATB1	70	31	79	31	75	34
KATB9	63	26	103	50	83	43
KATX69	63	26	69	21	66	25
M'sole	73	34	78	30	76	36
Masindi Yellow Short	71	32	90	40	80	40
NABE15	74	35	70	22	72	31
NABE16	77	37	108	53	93	51
NABE19	78	38	85	37	82	41
GLP585	88	45	70	22	79	39
FLOR DE MAYO	87	44	92	42	90	48
RWR 719	85	43	84	36	84	44

CT: cooking time

3.5.3 Variability in water absorption of 152 bean genotypes

The analysis of variance designated no significant differences in water absorption among the genotypes (Table 5).

Table 5: Mean squares for water absorption (% hydration) among the bean genotypes evaluated over two seasons in Kawanda

SOV	D.F.	M.S.	V.R.	F pr.
Genotype	151	86.7	0.7	1.0
Season	1	16.2	0.1	0.8
Genotype. Season	151	176.4	1.1	0.3
Residual	150	160.2		
SED	13.3			
GM	94.6			

*SED – standard error of difference, DF = degrees of freedom, SOV = source of variation, M.S = mean square, V.R = variance, F PR = f probability

Among the 152 genotypes, water absorption ranged from 63 % to 137 %, with an average of 94%. Ngwin x CAB2 had the least hydration capacity whereas it cooked for 56 minutes on average, considered intermediate cooking time. NABE15 had the highest hydration capacity of 137% although it was among the longest cooking genotypes (72) minutes. Among the short cooking genotypes, Awash melka, CNF5520, CAB2 had a hydration capacity of 98%, 87% and 98%. On the other hand, the longest cooking genotypes VAX4, KATX56, RWR3006 and RWR2154 had a hydration capacity of 99%, 89%, 104% and 97% respectively.

3.5.4 Variability in Iron and Zinc content in the genotypes

The analysis of variance (Table 6) showed that the genotypes differed significantly for Fe and Zn seed content. The genotype effect was highly significant ($P \leq 0.001$) for both seed Fe and Zn contents in the bush type but non-significant for climbers. Season effect was only significant for Fe concentration in bush beans, though effect of replications within seasons resulted to be significant for both Fe and Zn in bush and climber. However, the interaction between genotype

and season was not significant for both bean types. The analysis was done on plot basis hence the replications ensured good experimental precision.

Table 6: Mean squares for Iron and Zinc content in bean genotypes evaluated over two seasons at Kawanda

Source of variation	DF	Bush		DF	Climbers	
		Fe	Zn		Fe	Zn
Season	1	11347*	330	1	491	1124
Rep(Season)	4	1144***	846***	4	334*	95**
Genotype	120	308***	43***	30	151	20.7
Genotype. Season	122	1750	6.9	30	69	21
SED		3	2		5	1
%CV		6	5		8	4

Ns=non-significant, ** =significant at P=0.01 and ***= significant at P=0.001, % cv- coefficient of variation (percentage)

For season 2015B, Fe content ranged from 46-88 mg/kg, with an average of 64 mg/kg while Zn content varied at 24-40 mg/kg with an average of 30 mg/kg. During the season 2015D (September –December), Fe content ranged from 39-75 mg/kg with an average of 58 mg/kg while Zn content was between 26-42 mg/kg with an average of 33 mg/kg.

Across the two seasons, the 152 common bean genotypes used showed a wide variability in both the Fe and Zn content (Figure 2). The Fe content ranged between 39-86 mg/kg with an average mean of 60 mg/kg for bush beans while the Zn content ranged between 24-40 mg/kg with an average of 31mg/kg. A total of 61 bush genotypes had Fe content above the universal high Fe check (RWR2154) which had an average of 69 mg/kg. On the other hand, Fe and Zn seed content among the climbers ranged between 46-88 mg/kg with an average of 66mg/kg for Fe while Zn ranged between 27-42 mg/kg and average of 34 mg/kg (Table 7). Two genotypes (Nakaja and CAB2) had Fe content higher than the universal high Fe check (MIB 465) which had a concentration of 80 mg/kg for the first season while Nakaja (79 mg/kg) performed same as the check (MIB 465 -79 mg/kg) (Table 7). Across the seasons, the genotypes varied in the micro nutrient concentration. The perceptible changes were decreased Fe content and increased Zn content in season 2 (Table 8).

A

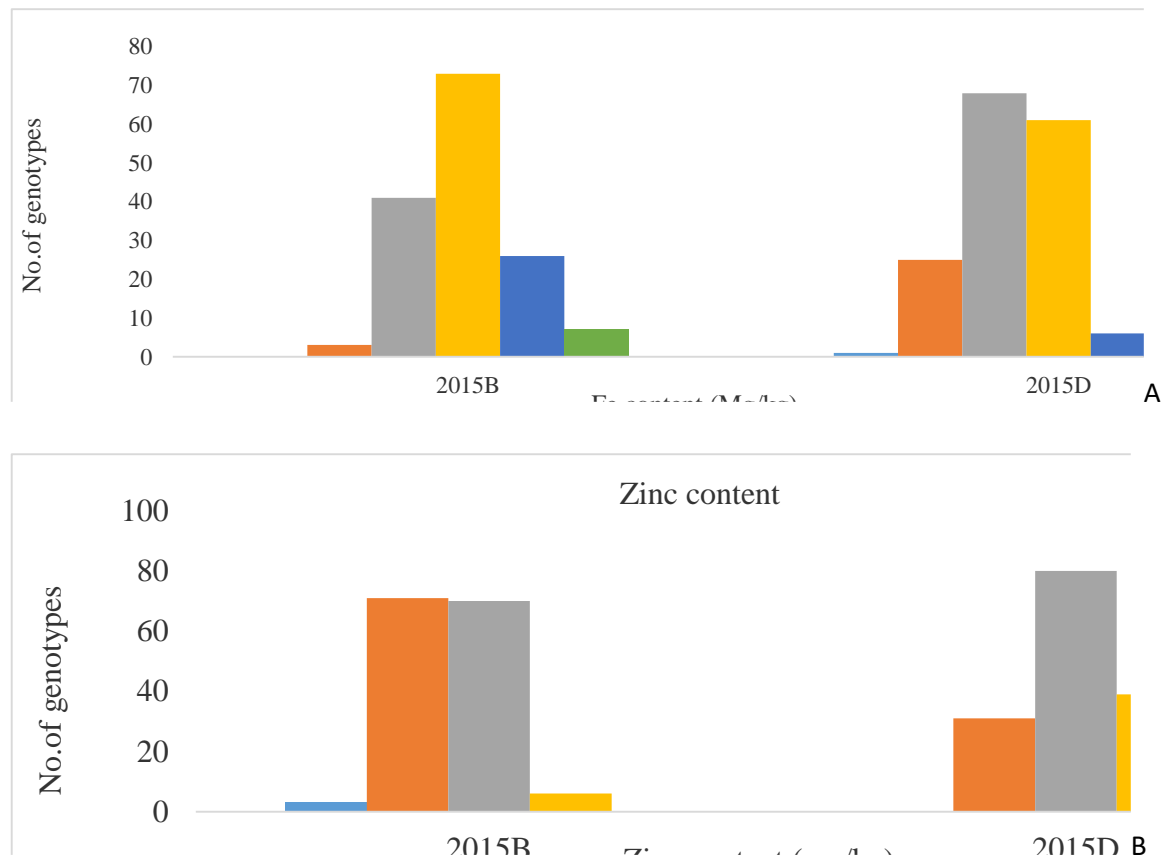


Figure 2: Average Iron (A) and Zinc (B) content in the evaluated common bean genotypes over two seasons

Table 7: Performance of evaluated bean genotypes for seed Iron and Zinc concentration with comparison to the iron check

Bean type	No. of genotypes	Season	Fe range (ppm)	Zn range (ppm)	Fe content of high Fe check	No. of genotypes with Fe > high Fe check
Bush	121	2015B	46-86	24-37	81 (RWR2154)	2
		2015D	39-75	26-40	59 (RWR2154)	59
Climber	31	2015B	55-88	27-40	85 (MIB465)	1
		2015D	46-75	30-42	74 (MIB465)	1

*Ppm= parts per million

Table 8 : Seed Iron and Zinc concentration in selected bean genotypes per season

Genotype	Micronutrient content					
	2015B	2015D	Average Fe	2015B	2015D	Average Zn
	Fe	Fe		Zn	Zn	
ACC714	86	75	81	33	35	34
NAKAJA	84	75	79	34	37	36
MIB 465	85	74	79	35	40	37
JESCA	84	72	78	34	37	35
CAB 2	88	66	77	38	38	38
VAX5	80	69	74	31	33	32
VAX1	73	74	74	34	39	37
Mexico 142	79	67	73	33	37	35
VAX2	77	67	72	32	34	33
AND620	74	69	71	35	40	38
RWR 719	73	68	71	32	36	34
VCB81013	73	68	71	33	42	38
GITANGA	71	70	71	32	40	36
MCM 2001	71	69	70	30	32	31
RWV3006	80	59	70	40	30	35
NABE29C	74	65	69	31	37	34
NABE3	72	67	69	30	34	32
RWR2154	81	57	69	34	32	33
NABE26C	72	65	69	31	38	35
VAX6	74	63	68	32	32	32
CNF5520	73	63	68	36	40	38
RWR 2245	70	67	68	31	37	34
NABE22	71	64	67	37	36	37
MAC 44	71	63	67	29	36	32
Awash melka	69	64	67	29	32	30
A344	71	62	67	32	36	34
ROBA-1	70	62	66	32	33	33
BIHOGO	70	60	65	33	35	34
SAB 686	69	56	63	31	34	32
Kanyebwa	57	49	53	26	28	27
KATB9	56	45	51	28	28	28
NUA8	53	48	51	24	29	27
NABE20	52	46	49	26	30	28
Mean	72.8	63.5	68.1	32.0	34.8	33.4
SED	7.9	7.5	7.2	3.2	3.6	3.0

* Names in bold indicate genotypes with high Fe and short cooking time, SED= standard error of difference

3.5.5 Variability of the genotypes in agronomic, yield and disease data

The genotypes were significantly different in terms of growth habit including germplasm with type I, type II, type III and type IV. The plant vigor demonstrated by the genotypes was also significantly different ranging from 1 = excellent to 3 = good, though the scale is up to 9 = very poor. The yield estimate from the genotypes was significantly different at $P=0.05$ ranging from 52 g (KATX56) to 1561 g (Twungurumirwango). The genotype yield effect was non - significant across seasons, as well as the disease response among the test genotypes (Table 9). Apart from the anthracnose in the bush beans, and the angular leaf spot in the pods, all the other diseases scored were not significant at $P=0.05$. Generally, the genotypes were not significantly attacked by the diseases in both the seasons this indicated a possibility of low disease pressure or good disease tolerance among the test genotypes.

Table 9: Mean squares for agronomic, diseases and yield data for the 152 evaluated bean genotypes

Trait	Bush		Climbers	
	Genotype MS	Gm	Genotype MS	Gm
GH	1.41***	2	3.36*	4
Plant vigor	405.56***	2	101.2*	3
Clean yield	257956.7***	493	254652.8***	598
BCMV	110.7ns	1	0.10 ns	2
CBBFL	126.5ns	2	0.93 ns	3
CBBFP	125.3ns	2	0.57 ns	2
RUSTFL	126.8ns	1	0 ns	2
RUSTFP	133.3ns	1	0 ns	2
ANTFL	0.43***	1	0 ns	1
ANTFP	0.39***	1	0 ns	1
ALSFL	1.2ns	2	0.64 ns	3
ALSFP	0.39***	2	0.29 ns	2

Ns=non-significant, ** =significant at $P=0.01$ and ***= significant at $P=0.001$. MS = Mean square, Gm = grand mean, GH = growth habit, BCMV = bean common mosaic virus, CBBFL = common bacterial blight in the field on leaves, CBBFP = common bacterial blight on field on the pods, RUSTFL = rust in field on leaves, RUSTFP = rust in field on pods, ANTFL = anthracnose in the field on leaves, ANTFP = anthracnose on pods, ALSFL = angular leaf spot in field on leaves, ALSFP = angular leaf spot in field on pods

3.5.6 Correlation of cooking time, %hydration, Fe and Zn content, diseases and yield

A strong positive correlation existed between Fe and Zn content, $r = 0.71$ ($P < 0.001$). Nevertheless, the relationship between cooking time and Fe and Zn was negative (-0.04 and 0.04 , respectively) (Table 10). A negative non-significant correlation of $r = -0.02$ was realized between percentage hydration and the cooking time. Some diseases were significantly correlated with others, a significant correlation between angular leaf spot in the leaves (ALSFL) with Bean common mosaic virus (BCMV) at $r = 0.4$ and common bacterial blight on leaves (CBBFL) $r = 0.45$. Similarly, BCMV was significantly correlated to CBB both on leaves and pods ($r = 0.56$ and $r = 0.29$) respectively, while CBB on the leaves was highly correlated to CBB on the pods ($r = 0.52$).

Table 10: Correlation of cooking time, Iron and Zinc, diseases and yield

	% hydration	CT	Fe	Zn	ALSFL	BCMV	CBBFL	CBBFP	RUSTFL
%hydration	-								
CT	-0.02	-							
Fe	-0.03	-0.04	-						
Zn	-0.08	0.04	0.71***	-					
ALSFL	-0.06	0.03	0.12	0.11	-				
BCMV	-0.24	0.17	0.24	0.34***	0.4***	-			
CBBFL	-0.06	0.15	0.04	0.17	0.45***	0.56***	-		
CBBFP	-0.20	0.02	0.00	0.05	0.26	0.29***	0.52***	-	
RUSTFL	-0.01	0.00	-0.14	-0.11	0.02	-0.10	0.07	0.06	-
Clean yield	-0.08	-0.06	0.4***	0.20	0.09	0.26	0.06	0.02	-0.13

*** = $P < 0.001$. -1: strong negative, +1: strong positive relationship, ***= significant at $P = 0.001$., CT = cooking time, Fe = Iron, Zn = Zinc, BCMV = bean common mosaic virus, CBBFL = common bacterial blight in the field on leaves, CBBFP = common bacterial blight on field on the pods, RUSTFL = rust in field on leaves, ALSFL = angular leaf spot in field on leaves

3.6 Discussion and conclusion

This study was done to identify common bean genotypes with short cooking time, high Fe and high Zn content. The genotypes evaluated showed a great diversity for these traits. Cooking time ranged from 35 to 100 minutes during the first season and between 43 to 122 minutes during the second season. Across the two seasons, the average cooking time was 66 minutes. Seasonal differences might have resulted from variations in the amount of rainfall, temperature and relative humidity. Cultivated under rain fed conditions the crop requires a minimum of 400 to 500 mm of rain during the growing season, but an annual total of 600 to 650 mm is considered ideal (Carbonell et al., 2003). The first season experienced a higher amount of rainfall (690 mm) as compared to second season (489 mm). The genotype by season interaction was significant ($P < 0.001$) for cooking time implying a contribution of seasons to differences in cooking time. This interaction may be explained by the possibility of interference of environmental conditions with genotypes in alteration of the seed tegument integrity, resulting in changes in their ability for water absorption and cooking time (Carbonell et al., 2003). Zilio et al., (2014) showed that temperatures lower than 30°C and air humidity higher than 40% during grain filling is ideal for lower cooking time in beans. Cooking time is also affected by the amount of rainfall per season. The first season had higher rainfall hence short cooking time among the genotype unlike the second season where the cooking time increased among the genotypes.

The hydration capacity for the genotypes did not vary significantly among the genotypes and within seasons, seed hydration capacity is known to be a function of the genotype and environmental conditions during development (Zilio et al., 2014). Water deficiency and high temperatures (around 30°C) between flowering and grain filling result in development of hard grain shells with low hydration capacity. A negative correlation ($r = -0.02$) was observed between percentage hydration and cooking time, which indicated that long cooking beans imbibed less water than fast cooking beans. However, this is contrary to the positive correlation ($r = +0.51$) reported by Dalla et al., (2003) and Bordin et al., (2010). These contradicting results could be well ascertained by developing a standardized method to determine the hydration capacity for grains before cooking (Bordin et al., 2010). The water absorption capacity of the bean seeds tend to be controlled by the seed coat texture among other factors (Shellie and Hosfield, 1991). The hard seed coat trait, therefore, affects the assessment of genetic potential for

cooking time. Different genotypes have different seed coat textures, which requires evaluation independently. When seeds have a moisture content of 9%, the hard seed coat problem is easily detected; but when moisture content is 12% or more, all genotypes tend to absorb similar amounts of water and therefore, the hard seed coat problem is not effectively detected. For this study, the cooking time evaluation was done at 12-13% seed moisture content hence the differences in water absorption were highly associated with genotypes seed coat texture. Differences in bean seed micropyle orifice dimension, the presence and number of seed coat pores and the microstructural differences are the major determinants of water uptake capacity of bean seeds (Agbo et al., 1987). According to Shellie and Hosfield (1991), when beans are cooked, native protopectin within the middle lamella forms a soluble pectin that depolymerizes rapidly during heating and allows water to quickly enter and migrate throughout cotyledonary cells. A high state of cellular hydration and heating thus allows cells to soften and separate. Reduced imbibition and/or compositional differences in pectin could be major factors affecting cooking time.

Among the total genotypes evaluated for Fe and Zn content, a total of 61 bush bean genotypes had Fe content higher than the high Fe check (RWR 2154) which had a mean content of 70 mg/kg. One hundred and one (101) genotypes performed better than the low Fe check CAL 96 (55 mg/kg) for the bush bean. Two genotypes of the climbing beans (Nakaja and CAB2) performed better than the high Fe check (MIB465) with a mean of 79.5 mg/kg. In general, the climbers had a higher Fe concentration than the bush bean type for the two seasons. This is associated with the longer days taken in the field by climber beans denoting the differences in the uptake and loading of Fe and Zn in common bean (Mukamuhirwa et al., 2015). However, the climbers did not show any significant differences for genotypes and genotype by season interactions. The days to maturity for climber beans are not significantly different, resulting to non-significant micronutrient uptake (Katungi et al, 2009).

In this study Fe and Zn showed a strong positive correlation ($r=0.71$) supporting the findings of Nchimbi-Msolla and Tryphone (2010) and Mukamuhirwa et al.,(2015). The highly significant positive correlation between these micronutrient concentrations in bean seeds suggests that genetic factors that increase Fe concentration co-segregate with genetic factors that increase Zn concentration. Selecting for bean seeds with high concentration of either Fe or Zn may, therefore, facilitate concentration of both elements (Nchimbi-Msolla and Tryphone, 2010).

A total of 15 genotypes (Amahunja, Awash melka, Bihogo, CAB 2, ECAPAN021, G858, Icaquimbaya, KK20, NABE12C, NABE4, NABE6, ROBA-1, RWR1873, RWV3006) were consistent in short cooking time for the two seasons and had a Fe content above the low Fe check (CAL96 – 55mg/kg). These comprise both climbers and bush beans as well as large and small seeded genotypes, thus, the combination of these traits in a genotype did not point to any particular direction, either by type, seed size or color.

In conclusion, the wide genetic diversity observed in this study for cooking time, Fe and Zn content supports the occurrence of diversity among genotypes in the greater Eastern Africa region; and therefore existence of alleles for bean improvement for nutritional traits (Asfaw et al., 2009). Great variability among genotypes allows selection for reduced cooking time and high micronutrient content.

Diversity analysis highlighted the potential of selected genotypes for use as parental materials in studies to understand the genetic control of cooking time, Fe and Zn content, and to breed fast cooking beans with high Fe and Zn content. These parents include the shortest cooking variety Awash melka (35 minutes) and the longest cooking variety VAX4 (100 minutes). For Fe and Zn, based on the concentrations of >70 mg/kg and >30mg/kg, ACC714, NABE3 and VAX4 presented high mineral concentration.

Iron and Zn showed a strong positive correlation. In this study, indirect selection of Fe by targeting Zn is possible (Mukamuhirwa et al., 2015; Ribeiro et al., 2013) since Zn seems to be the more reliable mineral to target in micronutrient improvement. However, the poor correlation between cooking time with both Fe and Zn makes indirect selection for these traits impossible, although some genotypes still have the desired combination of short cooking time and high Fe and Zn. Development of common bean genotypes with all three traits is, therefore, possible. A clear understanding of the inheritance patterns of these traits is, nevertheless, required to advise the selection of breeding methods.

CHAPTER 4

MODE OF INHERITANCE FOR COOKING TIME, SEED IRON AND ZINC CONTENT IN SELECTED COMMON BEAN GENOTYPES

4.1 Introduction

Short cooking time is of great importance in contributing to increased bean consumption, mainly by reducing the time and energy spent in meal preparation, and from the nutritional standpoint, for decreasing the loss of solids (Garden and McNeal, 2013). The nutritional requirements in alleviating micronutrient malnutrition particularly high Fe and Zn can be achieved through breeding for bio-fortified beans. Common bean is a major source of dietary protein for many people in Uganda. Bio-fortification will have a major impact on improving the quality of life for the majority of the population, and therefore needs to be addressed in breeding program (Broughton et al., 2003). Significant increases in the amount of bioavailable Fe and Zn in beans can be made through conventional plant breeding (Nchimbi-Msolla and Tryphone, 2010) based on the existing extensive variation of Fe and Zn content among bean genotypes. Genetic variability for seed cooking time and Fe and Zn content has been detected in common bean genotypes (Carbonell, 2003; Cichy et al., 2012). Therefore, selection of genotypes with high potential for use in breeding programs is possible.

Combination of short cooking bean varieties with high Fe and Zn content would go a long way in combating nutrition and energy usage challenges currently faced by consumers (Elia, 2003). Knowledge of the inheritance patterns expected for these traits is important in breeding for short cooking cultivars with high Fe and Zn content. The objective of this study was, therefore, to determine the mode of inheritance for cooking time, Fe and Zn content in common bean genotypes with the aim of developing bean varieties with decreased cooking time and high micronutrient content.

4.2 Materials and methods

4.2.1 Study site and germplasm

The study was carried out in the screen house at CIAT Uganda at the National Agricultural Research Laboratories (NARL) in Kawanda, Uganda from December 2015 to July 2016. Six genotypes bush bean were used as parental materials. These materials were selected from study one (Chapter three) based on their cooking time, Fe and Zn content (Plate 3; Table 11).



Plate 3: Bean parents used for inheritance studies for cooking time, seed iron and zinc content

4.2.2 Population development

The parents were established and crossed in 6 x 6 half diallel, including parents (Table 12) to explore all possible combinations and make inferences to the population on the nature and amount of genetic parameters, and GCA of parents and SCA of crosses. Crossing blocks were planted using a mixture of forest black soil, lake sand and decomposed farm yard manure in the ratio 3:1:1 currently used by CIAT. Four plants per parent were planted in 5 liter buckets, with

the same parents being planted weekly for four weeks. Four crossing blocks were set up, each with 10 buckets per parent. Watering was done in the mornings using a watering can. Routine management practices were carried out including fertilizer application NPK 17:17:17 using the rate of 5g per bucket at the base of the plant by hand before flowering. Hand weeding was done whenever the weeds appeared. Crossings were done after emasculating female parents and pollen from the male parent rubbed on the stigma. The bulk method was used to advance F_1 to F_2 . At least five F_1 seeds were obtained per cross and planted as described above. NPK fertilizer was applied (5g per bucket) to ensure maximum production in order to obtain enough F_2 seeds.

Table 11: Characteristics of ben parents used in inheritance study for cooking time, seed iron and zinc content.

Entry name	Origin	CT (minutes)	Average Mineral content (ppm)		Characteristics	Seed size and color	Gene pool
			Fe	Zn			
Awash Melka	Ethiopia	35	69	29	Short cooking time, low Fe	Small white	Mesoamerican
NgwakuNg waku	DRC	66	46	26	Intermediate cooking time, low Fe content	Medium yellow	Andean
CAL96	Uganda	39	54	26	Short cooking time and a Check for low Fe & Zn	Large Red mottled	Andean
KATX56	Kenya	91	51	28	Long cooking time and low in Fe	Large Red	Andean
VAX4	Uganda	100	66	31	Long cooking time	Small black	Andean
NABE3	Uganda	71	71	30	Long cooking time, high Fe	Small red	Mesoamerican

CT= cooking time, Fe= iron, Zn= zinc

Table 12: The mating scheme used for an inheritance study of cooking time, Iron and Zinc seed content at CIAT Uganda.

<div> PARENTS Male female </div>	Awash Melka	NABE3	CAL96	KATX56	VAX4	Ngwaku Ngwaku
Awash Melka	S					
NABE3	X	S				
CAL96	X	X	S			
KATX56	X	X	X	S		
VAX4	X	X	X	X	S	
NgwakuNgwaku	X	X	X	X	X	S

X= Cross, S= self.

4.3 Data collection and analysis

An average of fifty F₂ seeds were obtained per cross and bulked for each cross. These seeds were used in evaluations for cooking time, Fe and Zn content as described in chapter 3. Evaluations were carried out after two weeks from harvesting and at 12 % moisture content. Analysis of variance was conducted using Genstat computer package (12th Edition, VSN International Ltd. Copyright 2009).

4.3.1 Estimations of heritability

General and specific combining abilities were analyzed according to the fixed effect model I method II (Griffing, 1956) in order to estimate SCA and GCA effects for the hybrids and parents, respectively as: $Y_{ij} = \mu + GCA_i + SCA_{ij} + \text{error}$, where: μ =mean, GCA_i =the effect of male i, SCA_{ij} = the interaction effect of female i when crossed to j. Broad sense heritability was calculated as $H = V_G/V_P$, and narrow sense heritability, $h^2 = V_A/V_P$ where H and h^2 are broad sense heritability and narrow sense heritability respectively (Table 14), V_A is additive variance, V_G is genotypic variance and V_P is phenotypic variance (Hill et al., 2008).

Baker's ratios were used to predict performance of crosses based on GCA values (the importance of additive and non-additive gene effects) estimated as: $X = [2\sigma^2_{gca} / (2\sigma^2_{gca} + \sigma^2_{sca})]$, (Table 13) the closer the ratio is to 1 the greater the chances of predicting performance based on GCA (Baker, 1978).

Table 13: Formulae used for estimating heritability and Baker's ratio

	Formulae
Baker's ratio	$2\sigma^2_{GCA} / (2\sigma^2_{GCA} + \sigma^2_{SCA})$
NS-CGD (Genotype mean basis)	$2\sigma^2_{GCA} / (2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_e)$
BS-CGD (Genotype mean basis)	$(2\sigma^2_{GCA} + \sigma^2_{SCA}) / (2\sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_e)$

NS-CGD (Narrow Sense Coefficient of genetic determination) = Estimate of Narrow sense heritability (h^2) BS-CGD (Broad Sense Coefficient of genetic Determination) = Estimate of Broad Sense heritability (H).

4.4 Results

4.4.1 Performance of progeny and parents for cooking time, Fe and Zn content

The analysis of variance showed significant differences among crosses ($P < 0.001$) for cooking time, Fe and Zn content. General combining ability effects were highly significant ($P < 0.001$) for all three traits while the specific combining ability effects were also significant ($P < 0.001$) for Fe and Zn content as well as for cooking time ($P < 0.01$) (Table 14).

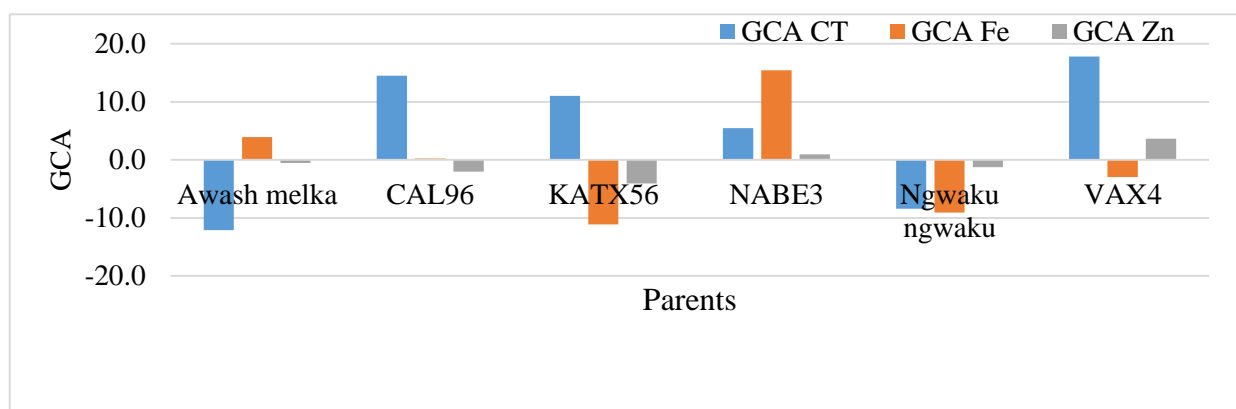
Table 14: Mean squares values for combining ability for cooking time, seed iron and zinc content in beans

	CT		Fe		Zn
Source	d.f	MS	d.f.	MS	
GCA	5	825.8***	5	274.1***	38.9***
SCA	11	429.5 **	9	17.7***	7.8***
Cross	13	553.4 **	14	109.23***	18.9***
Error	16	95.7	14	0.21	0.8

CT = cooking time, Fe = Fe, Zn = Zn, d.f = degrees of freedom, MS = mean squares, * $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

4.4.2 Estimates of general combining ability for cooking time, Fe and Zn seed content

Awash melka, and NgwakuNgwaku had significant negative GCA effects for cooking time, while the others had positive GCA's with VAX4 having the highest positive GCA. Awash melka, CAL96 and NABE3 had positive GCA for Fe while NABE3 and VAX4 had a positive GCA for Zn content (Figure 3). Awash melka combined desired negative GCA for cooking time and positive GCA for Fe though a low negative GCA for Zn. The best parent for short cooking time was Awash melka, while NABE3 was the best parent for high Fe content and VAX4 best for Zn content (Table 15).



GCA= general combining ability, CT= cooking time (minutes), Fe=iron, Zn= zinc (mg/kg)

Figure 3: General combining ability estimates for the evaluated parents for cooking time, seed iron and zinc content

Table 15: Mean square values for GCA effects and parental means for cooking time, seed iron and zinc content in beans

Parents	Parental means			GCA effects		
	CT	Fe	Zn	CT	Fe	Zn
Awash melka	26.0	58.95	32.75	-12.1**	5.5**	0.3 ns
CAL96	74.0	49.55	26	14.5***	1.8 ns	-1.0 ns
KATX56	52.0	40.1	24.75	11**	-9.6***	-3.3 **
NABE3	56.0	74	32.6	5.5 ns	17 ***	1.7 ns
NgwakuNgwaku	39.0	38.05	26.5	-8.4*	-7.5 ***	-0.5 ns
VAX4	102.0	52.95	36.55	17.8***	-1.4 ns	4.4 **
Mean	58.2	52.3	29.8			
SE				3.2	0.15	0.09

CT= cooking time, Fe =iron Zn= zinc, SE= standard error

4.4.3 Estimates of specific combining ability effects for the crosses

Specific combining ability effects for Fe content were positive for CAL96 x VAX4, NgwakuNgwaku x KATX56, NgwakuNgwaku x Awash melka, and NgwakuNgwaku x NABE 3. For Zn content, the positive SCA effects were observed in CAL96 x NABE3 and NgwakuNgwaku x KATX56. For cooking time, SCA effects were negative for Awash melka x VAX4 (P<0.05), CAL96 x NABE3 (P<0.01), NABE3 x KATX56 (ns), NgwakuNgwaku x VAX4 (P<0.01) and NgwakuNgwaku x KATX56 (P<0.05) (Table 16). NgwakuNgwaku x KATX56 combined the desired significant negative SCA for cooking time, with highly significant positive SCA effects for Fe and Zn. Depending on the seeds obtained per cross, cooking time or Fe and Zn content was determined. This accounts for the missing values.

Table 16: Mean square values for SCA effects

Cross	SCA effects		
	CT	Fe	Zn
Awash melka x KAT56	-5.3 ns	*	*
Awash melka x VAX4	-24.6 *	-0.2 ns	-0.6 *
CAL96 x NABE3	-36.4**	-1.8***	2.6***
CAL96 x VAX4	14.3 ns	6.5***	-1.6**
CAL96 x KATX56	*	2.8***	0.2 ns
CAL96 x NgwakuNgwaku	*	3.6***	3.2***
NABE3 x Awash melka	0.0 ns	*	*
NABE3 x VAX4	10.1*	5.8***	4.6**
NABE3 x KATX56	-8.4 ns	-0.5 ns	0.9 **
NgwakuNgwaku x Awash melka	6.6 ns	2.3***	1.4*
NgwakuNgwaku x KATX56	-20.2 *	5.2***	4.8***
NgwakuNgwaku x NABE3	8.5 ns	2.3***	-0.4 ns
NgwakuNgwaku x VAX4	-27.0 **	*	*
VAX4 x KATX56	8.3 ns	*	*
SE	8.7	0.4	0.25

*SCA=specific combining ability, CT = cooking time, Fe =Iron, Zn =Zinc, SE =standard error, ns = non-significant, * = P<0.05,

** = P <0.01. *** = P<0.001

4.4.4 Heritability estimates

Narrow sense heritability was estimated at 0.47, 0.89 and 0.71 for cooking time, Fe and Zn respectively. Broad sense heritability estimates were higher, at 0.94, 0.99 and 0.99 for cooking time, Fe and Zn respectively. Heritability estimated by Bakers ratio were closer to NSCGD values for the three traits (Table 17).

Table 17: Bakers ratio, Narrow sense heritability and broad sense heritability estimates

Trait	Bakers ratio	NSCGD	BSCGD
Cooking time	0.50	0.47	0.94
Fe	0.89	0.89	0.99
Zn	0.72	0.71	0.99

NSCGD = Narrow sense heritability coefficient of genetic determination, BSCGD =Broad sense heritability coefficient of genetic determination

4.5 Discussion and conclusion

The objective of this study was to determine the mode of inheritance for cooking time, Fe and Zn content in common bean genotypes. There were significant GCA and SCA effects for the three traits, signifying additive and non-additive gene effects contributed to the observed variation. This agrees with Elia (2003) on cooking time inheritance and Mukamuhirwa et al.,(2015) on Fe and Zn inheritance. The parental genotypes showed both significant and non-significant negative and positive GCA effects for cooking time, Fe and Zn content, indicating that both desirable and undesirable traits were acquired by the progeny from the parents for all the studied traits. Thus, importance of the interactions in determining the single cross progeny performance can be assessed by calculating the realtive importance of GCA to SCA.

The highest negative GCA effects significant for cooking time were shown by Awash melka (-12.1, $P<0.01$) and NgwakuNgwaku (-8.4, $P<0.05$); these were the short cooking parents (Table 16). These parents with highly significant GCA effects are considered good combiners and hence

desirable genotypes for use in breeding programs (Jacinto et al., 2003). Awash melka and NgwakuNgwaku were identified as good parents for short cooking time while VAX4, CAL96 and KATX56 were unsuitable for improvement of cooking time since they contributed to longer cooking time in their progenies.

On the other hand, positive GCA effects were desirable for Fe and Zn content as they indicate a larger genetic contribution to increased Fe and Zn content in the progenies (Mukamuhirwa et al., 2015). The highest significant positive GCA effects were displayed by Awash melka (3.4, $P < 0.01$) and NABE3 (15.5, $P < 0.001$). These parents also had intermediate and high Fe concentration at 58.9 and 74 mg/kg respectively. Similarly, positive GCA effects for Zn content were exhibited by NABE3 (0.9 ns) and VAX4 (3.6, $P < 0.01$). In the context of this study; to combine short cooking quality with high Fe and Zn content Awash melka would be considered a good parent although its contribution to high Zn content was not significant.

Among the 14 crosses evaluated, Awash melka x VAX4, CAL96 x NABE3, NgwakuNgwaku x KATX56, and NgwakuNgwaku x VAX4 showed significant negative SCA effects for cooking time. Notably, these crosses were between short cooking parents (Awash melka - 26 minutes, NgwakuNgwaku - 39 minutes) and a long cooking parent (VAX4 -102 minutes). This indicates strong action of the negative GCA parents and probable dominance of short cooking time over long cooking time as reported by Jacinto et al., (2003). This was also demonstrated by the bias of tested crosses in favor of the short cooking parent. The continuous distribution of cooking time indicates that it is a quantitative trait (Figure 1). However, Elia (2003) attributed skewness of the distribution in favor of short cooking parents to either maternal effects, or control by dominant genes.

The desired significant and positive SCA effects for Fe content were present in crosses CAL96 x KATX56, CAL96 x NgwakuNgwaku, CAL96 x VAX4, NgwakuNgwaku x Awash melka, NgwakuNgwaku x KATX56 and NgwakuNgwaku x NABE3. These crosses were from parents with positive GCA effects for Fe content hence showing them to be good combiners for Fe improvement in breeding. For Zn content, crosses CAL96 x NABE3, CAL96 x NgwakuNgwaku, NABE3 x KATX56, NgwakuNgwaku x Awash melka and NgwakuNgwaku x KATX56 (Table 17), had significant positive SCA effects for Zn content. These crosses could be considered for further advancement in breeding for increased Fe and Zn content respectively. However, in line

with this study, NgwakuNgwaku x KATX56 would be more promising crosses for further advancement and evaluation for development of short cooking bean genotypes with high Fe and Zn content.

In both field experiments, mean Fe and Zn concentrations in the genotypes showed continuous population distributions indicating quantitative inheritance for mineral content (Figure 2). In the present study, the narrow sense coefficient of genetic determination was observed at 0.47 for cooking time, 0.89 for Fe and 0.71 for Zn content. The broad sense coefficient of genetic determination was high at 0.94, 0.99 and 0.99 for cooking time, Fe and Zn respectively. This indicated that these traits are mainly governed by genetic factors as reported by Blair et al., (2010). The value of baker's ratio was relatively high for Fe (0.89), and Zn (0.71) indicating that additive gene effects are more important than non-additive gene effects in determining increase in Fe and Zn content as reported by Blair et al., (2009, 2010) and Mukamuhirwa et al.,(2015). The value of Bakers ratio for cooking time was midway (0.5) indicating importance of additive gene effects though non-additive gene effects are of considerable importance.

Studies by Elia (2003) and Jacinto et al (2003) reported narrow sense heritability at 0.9 and 0.76, respectively for cooking time. The observed differences in heritability values could have been due to population used. In this study F₂ seeds were used while Elia (2003) evaluated F₃ and F₄ seeds; and Jacinto et al (2003) evaluated F₆ and F₇ recombinant inbred lines. F₂ is a highly segregating population and is also highly heterozygous, with high linkage disequilibrium. The heritability for cooking time as observed in this study show that selection in early generations may not be effective.

In conclusion, since cooking time, seem to be quantitative trait controlled majorly by additive gene effects with considerable non-additive gene effects, it would be more effective to do many evaluations before selection of superior genotypes. Selection could, therefore, be effective in later generations, between F₄ and F₆. Selection for Fe and Zn content could be possible in early generations due to the higher magnitude of additive gene effect (81% and 71%) respectively. In addition, the progeny performance on cooking time, Fe and Zn may not be fully predictable based on parent performance alone. Simultaneous selection for short cooking time, high Fe and Zn could be possible by setting a selection index since both traits are quantitative.

From this study, the genotypes Awash melka and NgwakuNgwaku are recommended as parental materials for short cooking time; NABE 3 and Awash melka as high Fe parents; and Awash melka and NABE 3 for combining short cooking time with high mineral content. The cross NgwakuNgwaku x KAT X56 is also a promising cross to evaluate for short cooking time with high Fe and Zn content.

CHAPTER 5

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussions

This study was done to determine the genetic variability for cooking time, Fe and Zn content among common bean genotypes, and to understand the mode of inheritance for these traits. The study revealed a high level ($P < 0.001$) of genetic variations in cooking time, Fe and Zn content among the common bean genotypes. This study sought to exploit this diversity so as to provide valuable alleles for breeding for short cooking bean genotypes with increased Fe and Zn content.

A significant strong positive correlation ($r = 0.71$) was observed between Fe and Zn content in seeds of bean signifying that genetic factors that increase Fe concentration are co-segregating and co-localized with those that increase Zn concentration. Therefore, selecting for bean seeds with high concentration of either Fe or Zn may increase the amount of both elements. A weak correlation between cooking time and Fe and Zn $r = -0.04$ and 0.04 respectively was observed, suggesting that selection for cooking time should be done independently from the micronutrient content. A negative non-significant correlation was also observed between cooking time and hydration capacity ($r = -0.02$). This indicates the improbability of using water absorption as an indirect selection method for cooking time, though further studies should be done to establish a conclusive relationship.

Analyses of variance (ANOVA) for combining ability revealed that both additive and non-additive gene actions were important for cooking time, Fe and Zn content. Cooking time was shown to be a quantitatively inherited trait and primarily additive in nature based on high significant ($P < 0.001$) GCA obtained. This indicates that many genes with relatively smaller and accumulative effects are present in every parent to account for the variation of cooking time found in the F_2 progenies, hence the importance of additive gene action. However, the skewing of F_2 distribution in favor of the short cooking time parents may suggest that the trait is governed by dominant genes as reported by Jacinto et al.(2003).

The inheritance of Fe and Zn buildup in common bean seeds was shown to be predominantly quantitative. Transgressive segregation for lower mineral accumulation was most evident for

seed Zn as compared to Fe. The transgressive segregation observed in the Zn concentration population distributions could have resulted from the combination of Andean and Mesoamerican genes for mineral accumulation in the inter gene pool crosses evaluated. Introgressed genotypes between Andean and Mesoamerican are likely to be higher in Fe and Zn than non-introgressed Andean genotypes (Blair et al., 2010). Introgressed genotypes tend to utilize the micronutrient differences in the two gene pools. The Andean beans tend to have higher Fe content but low Zn, while the Mesoamericans have high Zn content (Blair et al., 2010).

Heritability values for Fe and Zn content were high (0.89 and 0.72 respectively). This indicates the possibility of early generation selection for these traits. However, selection at an advanced stage would be more beneficial for cooking time due to the low heritability (0.5). More studies to establish a concrete heritability would be of great contribution. The broad sense coefficient of genetic determination was high for all the traits (0.94, 0.99 and 0.99 for cooking time, Fe and Zn respectively) indicating high genetic control for the traits. This shows that selection to shorten bean cooking time and increase the micronutrient (Fe and Zn) concentration is highly possible because the greatest proportion of variation observed is due to genetic factors.

Awash melka and NgwakuNgwaku displayed more significant GCA for cooking time implying that they transfer the short cooking time effectively when crossed with other genotypes. NABE3 and Awash melka had the desired positive GCA for Fe content, implying that these parents could be useful as donors to transfer high Fe content to their crosses.

5.2 Conclusions

The findings of this study clearly show the potential to obtain short cooking common bean genotypes with improved Fe and Zn content as well as other farmer preferred traits. Breeding for increased Fe and Zn content in commercial cultivars could be done as a first step, followed by breeding for reduced cooking time; alternatively, both traits could be bred for simultaneously by use of selection indices. Increasing Fe and Zn concentration in common bean germplasm can be targeted in early generations as opposed to short cooking time which has a relatively low heritability value, in addition, micronutrient concentration was less variable across seasons as opposed to cooking time.

5.3 Recommendations

It is recommended that parental genotypes Awash melka and NgwakuNgwaku which displayed high negative GCA effects for short cooking time be used for improving this trait in the market-class dry beans in Uganda, while NABE 3 is a recommended parent for Fe content improvement.

From the heritability values attained and the correlations of cooking time to the mineral content, it would be more advantageous to target breeding for these traits separately. The heritability of these traits varies and depends on the type of population and the environment to which the genotypes are subjected to. It is recommended that characteristics of short cooking time, Fe and Zn should be investigated in every new parental source upon initial introduction into to a breeding programme

The stability of cooking time, Fe and Zn of the selected promising genotypes from Uganda should be evaluated across environments. Additional studies on combining ability and mode of inheritance of bean genotypes for the three traits evaluated in this study would support identification of the best parents for use in breeding for short cooking time and high Fe and Zn content.

The cooking time experiment was carried out for a period of sixty days for evaluation of 350 samples. With a larger sample size, more time could have been utilized, thus there is need for identification of markers associated with the trait as a means of indirect selection .This might increase selection efficiency.

The relationship between water absorption and cooking time especially after storing been seeds for a period of time (one month) should be further investigated. A positive relationship may enable use of water absorption as an indirect indicator for selection of cooking time which is a cheaper and faster alternative compared to cooking time.

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APPENDIX

Appendix A: Summary table of means agronomic, cooking time, iron and Zn content, %hydration, yield and disease data for the 152 genotypes evaluated for two seasons at Kawanda

Entry name	GH	DF	CT	Fe	Zn	%hydration	Clean yield	ALSF	BCMV	CBBFL	CBBFP	RUSTFL
A197	1	34	74	53	26	95	405	2	1	2	2	1
A222	2	35	63	64	28	98	1089	2	1	2	2	1
A286	1	40	60	62	31	95	373	2	1	2	2	1
A344	1	37	65	66	34	88	465	2	1	2	2	1
AB136	4	42	64	63	33	96	502	3	2	3	2	1
ACC714	2	40	65	81	34	90	324	2	1	2	2	1
AFR-703	1	36	56	55	28	91	318	2	1	2	2	1
AFR708	1	35	78	60	29	101	348	2	1	2	2	1
AKARYOSE	1	33	51	61	35	85	160	2	1	2	2	1
AMAHUNJA	3	44	47	65	35	102	1147	2	1	2	2	1
AND 1062	2	38	92	62	32	99	921	2	1	2	2	1
AND10	1	36	56	61	32	91	689	2	1	2	2	1
AND277	1	33	58	57	32	97	354	2	1	2	2	1
AND279	1	35	55	57	29	102	471	2	1	2	3	1
AND620	2	40	58	71	38	95	415	2	1	2	2	1
Awash 1	2	35	55	59	32	95	603	2	1	2	2	1
Awash melka	1	40	43	67	30	88	491	2	1	2	2	1
BALONBAYO 11796	4	33	45	*	*	104	*	2	2	2	2	1
BAT332	1	40	87	60	32	97	744	2	1	2	2	1
BIHOGO	3	45	49	65	34	94	555	2	1	2	2	1
BISERA	1	33	58	64	30	98	320	3	1	2	3	1
CAB 2	4	45	43	77	38	98	393	3	2	3	2	1
CAL123	1	38	53	64	33	92	359	2	2	2	2	1
CAL143	1	35	58	62	29	83	401	2	1	2	2	1
CAL96	1	34	57	55	27	91	230	2	1	2	2	1
CNF5520	3	40	42	68	38	87	940	2	1	2	2	1
CODMLB001	1	34	64	55	31	92	397	2	1	3	2	1
CODMLB033	1	34	55	62	29	103	679	2	1	2	2	1
Cornell 49-242	1	41	79	59	34	101	478	2	1	2	2	1
DECELAYA	4	45	61	58	34	95	614	3	2	3	2	1
DON TIMOTEO	3	39	67	57	32	98	578	2	1	2	2	1
DOR 500	1	40	88	61	33	83	806	2	1	2	2	1
DRK 64	2	41	57	*	*	90	1168	2	1	2	2	1
ECAPAN021	3	39	53	63	35	96	788	2	2	2	2	1
FLOR DE MAYO	4	43	90	62	36	89	272	3	2	3	2	1

G21212	2	38	68	66	33	85	527	2	2	2	2	1
G2333	4	43	58	56	33	94	767	2	2	3	3	1
G2858	3	38	54	63	30	99	936	2	1	2	2	1
G5686	1	35	71	50	30	94	253	2	1	2	2	1
G685	4	46	59	65	33	89	720	3	3	4	2	1
G858	4	45	51	63	34	92	951	2	2	2	2	1
GASIRIDA	4	45	56	61	31	81	504	2	2	2	2	1
GITANGA	4	42	63	70	36	91	260	2	2	3	2	1
GLP2	1	39	62	58	31	94	564	2	1	2	2	1
GLP585	4	43	79	62	35	91	232	2	2	3	3	1
HM21-7	1	35	62	53	31	90	391	2	1	3	2	1
ICAQUIMBAYA	1	34	52	*	*	90	415	2	1	2	2	1
Inamuhire	1	32	79	52	30	84	330	2	1	2	2	1
JESCA	3	41	64	78	35	93	1291	2	1	2	2	1
Kanyebwa	1	32	91	53	27	91	345	2	1	3	3	1
KATB1	1	31	75	56	31	100	136	2	1	2	2	1
KATB9	1	32	83	51	28	98	268	3	1	2	2	1
KATX56	1	32	99	48	30	89	52	2	1	2	2	1
KATX69	1	32	66	56	31	105	*	3	1	2	2	2
KK20	3	39	53	57	30	96	716	2	1	2	2	2
KK8	2	40	63	61	27	96	820	2	1	2	2	1
M'sole	2	34	76	64	33	90	607	2	1	2	2	1
MAC 42	4	42	62	61	34	85	272	2	2	2	2	1
MAC 44	4	43	60	67	32	77	978	3	3	3	3	1
Maharage Soja	2	38	64	69	30	90	857	2	1	3	2	1
MASINDI YELLOW LONG	2	33	62	57	30	97	239	2	1	2	2	1
MASINDI YELLOW SHORT	1	32	80	54	31	90	116	3	1	2	2	2
MCM 1015	1	40	56	64	31	94	610	2	1	2	2	1
MCM 2001	2	40	62	70	31	76	648	3	1	2	2	1
MCM 5001	1	41	79	62	30	95	710	2	1	2	2	1
Mexico 142	2	41	60	73	35	93	341	2	1	2	2	1
MEXICO 54	4	43	68	59	34	84	259	2	2	3	2	1
MIB 465	2	39	70	79	37	99	610	2	1	2	2	1
Michelite	2	36	61	67	36	91	135	2	1	3	3	1
MLB-49-89A	4	38	64	67	36	75	330	3	2	4	4	1
MONTCALM	1	33	59	55	32	97	292	2	1	2	2	1
MUKUNGUGU	2	35	69	67	33	101	383	2	1	2	2	1
MUSENGO	2	36	56	66	34	98	531	2	1	2	2	1
NABE1	1	35	62	51	28	104	291	3	1	2	2	1
NABE10C	4	43	58	59	33	97	856	3	2	4	3	1
NABE11	1	33	64	67	35	100	222	2	1	2	2	1

NABE12C	4	42	51	65	34	95	1175	3	2	3	3	1
NABE13	1	34	61	54	32	101	615	2	1	2	2	1
NABE14	1	34	61	63	33	99	456	2	1	2	2	1
NABE15	1	31	72	52	27	137	191	2	1	3	2	1
NABE16	2	33	93	52	27	91	262	2	1	2	2	1
NABE17	2	32	62	54	29	97	200	2	1	2	2	1
NABE18	1	36	84	54	28	89	245	2	1	2	2	1
NABE19	2	34	81	46	27	92	232	2	1	2	3	1
NABE2	1	41	66	65	30	102	588	2	1	2	2	1
NABE20	2	33	70	49	28	83	257	2	1	2	2	1
NABE21	1	33	77	54	32	102	217	2	1	2	2	1
NABE22	1	34	58	67	37	109	105	2	1	2	2	1
NABE23	1	33	62	65	31	94	260	2	1	2	2	1
NABE26C	4	45	55	69	35	97	780	2	2	2	2	1
NABE29C	4	45	66	69	34	98	727	2	2	2	2	1
NABE3	2	39	90	69	32	95	725	2	1	2	2	1
NABE4	1	35	52	58	32	91	238	2	1	2	2	1
NABE5	2	36	62	60	35	90	265	2	1	2	2	1
NABE6	1	41	46	63	34	96	*	9	1	2	2	1
NABE7C	4	42	63	58	31	93	1118	3	2	3	2	1
NABE8C	4	43	91	59	31	102	253	2	2	3	2	1
NABE9C	4	43	50	61	33	86	322	2	2	2	2	1
NAKAJA	3	41	62	79	36	96	1365	2	2	2	2	1
NGWAKU NGWAKU	1	33	84	43	27	98	298	2	1	3	2	1
NGWIN X CAB 2	4	48	56	64	34	63	898	2	2	3	3	1
NUA35	1	36	71	54	27	93	239	2	1	2	2	1
NUA45	2	37	82	55	29	99	252	2	1	2	2	1
NUA59	1	34	65	63	29	91	560	2	1	2	2	1
NUA8	1	33	57	51	26	91	387	2	1	2	2	1
NUA99	1	33	60	63	30	100	524	2	1	2	2	1
PAN150	2	39	60	69	33	108	356	2	1	2	2	1
PI 207262	2	37	61	71	37	90	541	2	1	2	2	1
RANJONOMBY	1	35	104	51	30	104	256	2	1	2	2	1
ROBA-1	2	41	52	66	33	101	939	3	1	2	2	1
RWR 1092	2	34	90	61	34	88	399	2	1	2	2	1
RWR 1873	1	35	48	58	31	98	474	2	1	2	2	1
RWR 2091	1	35	69	57	30	90	774	2	1	2	2	1
RWR 2245	2	33	67	68	34	94	441	3	1	2	2	1
RWR 719	2	40	84	71	34	99	701	3	1	3	2	1
RWR1180	1	38	102	61	31	117	467	2	1	2	2	1

RWR2154	1	34	94	69	33	97	616	3	1	2	2	1
RWR362	1	34	49	56	30	99	577	2	1	3	2	1
RWV1129	3	41	51	69	34	99	602	3	2	3	3	1
RWV2887	4	48	58	58	31	101	867	3	2	3	2	1
RWV3006	4	43	50	70	35	104	419	3	2	3	2	1
RWV3316	4	40	75	68	34	93	247	3	2	2	2	1
SAB 620	1	32	82	52	28	89	193	2	1	2	2	1
SAB 622	1	33	57	47	28	85	376	2	1	2	2	2
SAB 626	1	36	59	56	31	102	791	2	1	2	2	1
SAB 629	1	34	61	56	32	102	272	2	1	2	2	1
SAB 630	1	33	66	60	32	93	124	2	1	2	2	1
SAB 650	1	33	64	53	28	80	195	3	1	2	2	1
SAB 659	1	33	69	62	34	92	378	2	1	2	2	1
SAB 686	1	33	71	63	32	92	186	2	1	2	2	1
SAB 712	1	33	60	59	30	101	190	2	1	3	3	2
SCAM-80CM/15	2	35	78	69	35	92	614	2	1	2	2	1
SEA 15	2	36	57	59	35	92	276	2	1	2	2	1
SELIAN 97	1	37	76	56	32	98	149	2	1	2	2	1
SER 16	1	36	62	55	31	96	519	2	2	2	2	1
SER 48	2	38	60	62	31	96	649	2	1	2	2	1
SER 82	2	34	59	58	31	87	726	2	1	2	2	1
TO	2	34	89	63	33	98	164	2	1	3	2	2
TU	2	34	62	66	36	96	435	2	1	2	2	1
TWUNGURUMIRWANGO	3	42	44	66	33	78	1561	2	2	2	2	1
UBR (92)25	1	37	69	64	34	101	404	2	1	2	2	1
URUGEZI	1	34	58	61	33	93	507	2	1	2	2	1
URWERA	1	32	67	59	30	100	198	2	1	2	2	1
VAX1	2	40	61	74	37	100	1013	2	1	2	2	1
VAX2	2	39	72	72	33	93	637	2	1	2	2	1
VAX3	2	40	55	58	30	96	841	2	1	2	2	1
VAX4	1	39	104	61	30	99	969	2	1	2	2	1
VAX5	1	39	67	74	32	98	332	2	2	2	2	1
VAX6	2	38	73	68	32	96	918	2	1	2	2	1
VCB81013	4	48	81	71	38	97	838	2	2	3	2	1
VT TT 923/10-3	2	38	70	65	33	88	346	2	1	2	2	1
ZEBRA	2	38	56	67	30	84	*	2	1	3	3	1

GH= growth habit, DF= days to flowering, CT=cooking time (minutes), Fe=iron, Zn= zinc, BCMV = bean common mosaic virus, CBBFL = common bacterial blight in the field on leaves, CBBFP = common bacterial blight on field on the pods, RUSTFL = rust in field on leaves, RUSTFP = rust in field on pods, ALSFL = angular leaf spot in field on leaves